THE

AMERICAN JOURNAL

OF

PHYSIOLOGY

VOLUME 137

CONTENTS

iii

CONTRACTION POTENTIALS (RIGHT QUADRICEPS FEMORIS) IN MAN DURING READING[1]

EDMUND JACOBSON AND FRANCIS L. KRAFT

From the Laboratory for Clinical Physiology and Wells College

In 1886, Mitchell and Lewis concluded that "the responsive jerk brought about by striking a stretched tendon is the most refined measure we possess of deciding as to the tone of muscle." Since that date, contraction in the quadriceps femoris, as tested by the knee-jerk, has been used most often and widely as an indication of functional as well as organic condition of the nervous system. For over half a century various investigators reported on such tests both in the lying and sitting postures and in a variety of occupations, including reading and writing (Lombard, 1887). However, the knee-jerk does not readily lend itself to precise quantitative determination, because of difficulties mentioned previously (Jacobson, 1928). Accordingly, in the present investigation on quadriceps activity during reading in the sitting posture, measurements are made of action-potentials—or, as we prefer to call them in this instance—contraction potentials.

One hundred unselected subjects, presumed to be in a fair state of health, were tested. They were 39 males and 61 females, ranging in age between 22 and 61 years, mostly employees in various business occupations.[2] The room was semi-sound proof and apparently free from apparatus, while unnecessary sounds, including conversation, were largely avoided. Electrodes were platinum iridium wires, 0.011 inch in diameter, inserted perpendicularly to a depth of 11 mm. Commonly the lower electrode was placed about two inches above the patella in the midline of the upper surface of the thigh, while the upper one was placed about two inches or a little more above this level. As stated previously, the continued presence of such fine wires is attended by no noteworthy subjective discomfort and does not act as a distractive. In these respects, as in others, electrical measurements have advantages over the older procedure of knee-jerk testing.

Recording is without photography, by means of the Integrating Myovoltmeter,

---

[2] Twenty-four were secretaries, 6 were college professors, 6 were dentists, lawyers or doctors, 9 were housewives, 3 were switchboard operators, 4 were advertising agents, 8 were clerks or accountants; the remainder were distributed approximately one per business occupation, differing widely from each other.

as described previously (Jacobson, 1940). This consists of an a.c. amplifier connected with a vacuum tube rectifier and other means for causing the amplified contraction-potentials to charge a condenser in such a way that the magnitude of the charge is a measure of the average contraction-potential for the charging period. At any instant the total rectifier output is indicated by the position of a needle on a meter. Fluctuations of this needle enable the investigator to follow the gross variations of contraction in the muscle studied; if the subject extends his leg, the needle moves up on the dial accordingly and stays up as long as the extension is maintained; but if the muscle is limp and relaxed, the needle remains near zero.

In seeking a quantitative indication of tonus in a muscle as a whole, we are led away from the employment of micro-electrodes and of concentric electrodes, which were found suitable in the investigation of the electrical activity of single fibers or of small groups of fibers discharging as a unit. If it were possible to place a pair of concentric electrodes in juxtaposition with each and every muscle fiber in the muscle group tested and so to measure each and every discharge, a plot of voltages and frequencies against time for all the fibers would be an ideal electrical measurement of contraction in the muscle group. Since this is beyond possibility, wire electrodes are inserted into the muscle tissue where an indefinite number of fibers discharge variously from instant to instant. Obviously, at any instant the p.d. in the electrodes is the resultant from discharges in the surrounding muscle mass. How far a fiber can be from such a wire and yet affect it thus, we do not know. At any rate, total electrification in the muscle is not measured, but only the potential differences in the two wires. To what extent, if any, the values vary with the size and length of the wires, the distance apart and their locality in the muscle is under investigation.

For quantitative purposes, it has seemed best to limit the frequency range measured. Accordingly, what we measure is a function, within limits, of the electrical activity of the many fibers which affect the two electrodes inserted with a view to obtain a sample of the total activity of the muscle.

In the present study, the voltages recorded during muscular contractions can be expected to be lower than those reported by most previous investigators of muscular action-potentials for three reasons: first, because the muscles here tested are in a state of slight contraction, not far removed from rest, while the muscles in experiments previously conducted by others generally have been stimulated to contract intensively; second, because a.c. amplifiers of limited frequency range have been developed and improved from time to time specifically to make possible these low voltage measurements in muscle; third, because at any instant under our conditions some fibers contribute positively, others negatively, determining a resultant p.d. in the electrodes which consequently is less than the voltage recorded when concentric or other electrodes are stationed approximately in contact with a single fiber; for then, a voltage builds up to a maximum (i.e., critical) value of discharge for that fiber whereupon a constant p.d. can be recorded each time that fiber contracts.

Each subject was requested to sit quietly and to read to himself. The right

foot hung freely, while the left rested on a support. The arms held the magazine, but were not provided with special supports. The rectified contraction-potentials were averaged and integrated every two minutes during a thirty minute period of test. No subject had been tested previously or had taken a sedative or had received any training to relax or knew the purpose of the study.
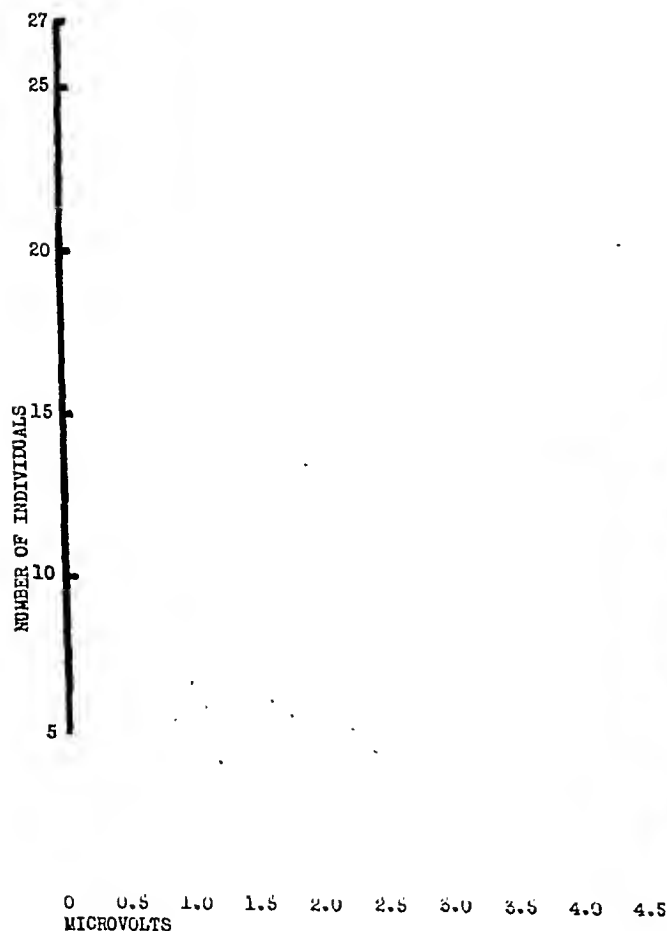
Fig. 1
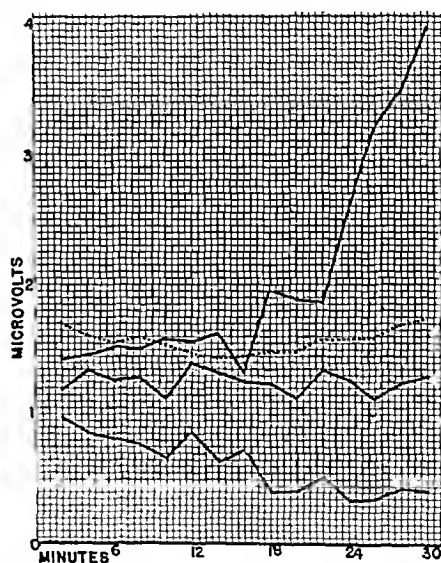
Fig. 2

Fig. 1. Distribution curve of average microvoltages for the thirty minute period of test in all subjects, except one.

Fig. 2. *Unbroken lines:* Contraction potentials plotted against time in three subjects, illustrating ascending, horizontal and descending curves. *Broken line:* Composite curve for 99 subjects. (In this figure each point plotted indicates the average microvoltage for the preceding 2 min.).

The results can be presented 1, as averages of the potential differences for the entire thirty minute period; 2, as curves of the integrated contraction-potentials plotted against time.

In figure 1, the distribution curve of the averaged potentials for the group is plotted on a 0.5 microvolt scale. Evidently the curve is normal in type. The values range from 0.5 to 4.5 microvolts with the maximum number of indi-

viduals (35) in the range from 1.0 to 1.5 microvolts. One subject (H) has been omitted, because his average (19.5) was considerably higher than that for the others and could not be included in the graph conveniently.

The curves representing the potentials plotted against time can be classified in three groups, although in many instances the distinction is doubtful or approximate only: 1, those on the whole showing a tendency toward increase of tension throughout the period—ascending curves; 2, those on the whole showing a tendency toward decrease of tension throughout the period; 3, those showing ups and downs but on the whole in no particular direction. Selected examples of types 1, 2 and 3 appear in figure 2. The curves are approximately descending in 26 instances, approximately ascending in 41 instances and more or less horizontal in 33 instances. Apparently the first group are more tense at the beginning of the period, tending to become adjusted as the minutes pass. Another group becomes more restless toward the end of the period, perhaps because of the necessity to sit still. The third group remains more nearly constant. Consideration of the three tendencies illustrated in figure 2 is of aid in the interpretation of the composite curve shown in figure 2.

Under the conditions of test, the course of the composite curve for 100 individuals over the thirty minute period (not shown in the figures) runs at all points between the values of 1.5 to 2.0 microvolts. The highest levels occur approximately from 0 to 2 and 26 to 30 minutes, while the lowest level occurs from 16 to 18 minutes. From a statistical standpoint, the composite curve would be more representative if the figures for subject H were omitted. When this is done, the result is shown in the broken line curve in figure 2. Throughout its course, it falls in value between 1.4 and 1.8 microvolts. Its highest points are at the first and final two minutes, respectively about 1.7 and 1.8. Its lowest point is approximately at the middle (12–14 min.). The explanation of the moderate concavity upwards of the composite curve evidently is that the higher values due to group 1 are reflected in the first portion of the curve, the lower values due to group 2 are reflected in the final portion of the curve, while the resultant of all three groups tends to bring the composite minimum toward the middle of the curve.

In some instances, following the thirty minute test reported above, an additional test was made during a five minute period, but in the lying posture with eyes closed. The subject was requested to relax. Some failed to relax, as measured by the voltages, but the ability did not always correspond with the performance while reading, as illustrated in the case of subject H. Upon lying down, the values for the five minute period were respectively 0.07, 0.15, 0.17, 0.14 and 0.13. These values fall toward the lowest levels commonly recorded for healthy subjects (not trained) (Jacobson, 1939). Yet, while reading, his values never fell below 9.8 and reached as high as 30. Evidently, ability to relax lying down does not necessarily indicate ability to relax when engaged in activities such as reading in the sitting posture, although a tendency toward carry-over has been noted in certain subjects upon training (Jacobson, 1942).

As interpreted in the light of previous investigations on the knee-jerk, descending curves would indicate progressive differential relaxation while ascending curves would indicate increasing contraction in the region measured. In this group of apparently healthy individuals, a distinct trend toward differential relaxation while reading is exhibited only by a minority.

CONCLUSION

A new method of measuring muscle potentials (potential differences in electrodes in muscle), plotting their values against time, is further illustrated. Direct electrical measurements are made of muscular contraction (tonus) in man in the right quadriceps femoris in 100 subjects engaged in silent reading under controlled conditions. The composite curve may serve as a standard for future investigations under these conditions.

REFERENCES

MITCHELL, S. W. AND M. J. LEWIS. Trans. Assn. Am. Phys. 1: 11, 1886.
LOMBARD, W. P. Am. J. Psychol. 1: 5, 1887.
JACOBSON, E. This Journal 86: 1, 15, 1928.
       Am. J. Psychol. 52: 620, 1939.
       Rev. Sci. Instr. 11: 12, 415, 1940.
       In press, 1942.

BLOOD ELECTROLYTE CHANGES IN THE HEART-LUNG PREPARATION WITH SPECIAL REFERENCE TO THE EFFECTS OF CARDIAC GLYCOSIDES[1]

E. H. WOOD[2,4] AND G. K. MOE[3,4]

From the Department of Physiology, University of Minnesota, Minneapolis

Knowledge concerning the electrolyte and water exchanges of striated muscle in association with activity has been greatly amplified and extended during recent years (1, 2). However, evidence concerning electrolyte exchanges of cardiac muscle as a result of variation in cardiac activity is for the most part indirect and inconclusive. Howell and Duke (3) demonstrated that vagal inhibition of the dog heart resulted in an increase in the potassium content of the perfusion fluid. This finding has been confirmed on the turtle heart (4). Kehar, McCollum and Hooker (5, 6) showed that ventricular fibrillation of hearts perfused through the coronaries caused a loss of potassium from the heart and a gain of water. Harrison and co-workers (7) considered the decrease in potassium content which they found in human "heart failure" hearts to be due to overwork of the cardiac muscle. Cardiac hypertrophy may be associated with a gain in cardiac water (8) and a loss of creatine, potassium, and phosphorus (9, 10). Cardiac hypertrophy in thyroid-fed rats apparently does not significantly alter the electrolyte composition of the heart (11, 12). There is indirect evidence that asphyxia causes a loss of potassium from cardiac muscle (13, 14). Herrmann (15) has failed to confirm this finding by means of direct tissue analyses. The evidence concerning cardiac electrolyte exchanges resulting from the action of digitalis glycosides has been cited (28).

The conflicting results concerning the effect of therapeutic doses of digitalis on cardiac potassium content (16, 17, 18, 19, 20) are probably for the most part due to the lack of sensitivity of the methods used to detect relatively small potassium exchanges. The usual method of paired muscle analyses is not applicable to the heart. The utility of direct tissue analyses is restricted therefore to the detection of relatively large exchanges since the range of variation in the electrolyte composition of the cardiac muscle from animal to animal is considerably greater than 10 per cent. The method of sampling the heart before and after experimental procedures (19) is open to numerous objections. These considerations lead to the conclusion that some method of determination of concentration changes in the perfusion fluid of the heart under physiological

conditions offers the best possibilities for the determination of relatively small cardiac electrolyte exchanges. The difficulties of carrying out such a study in an intact animal are considerable. The investigator is therefore forced to use some type of isolated heart preparation. Any preparation using saline as the perfusion fluid is far removed from physiological conditions. The nearest approach to a physiological, isolated heart preparation is the Starling heart-lung preparation. The above considerations led us to study the electrolyte and water equilibria in the heart-lung preparation. Some of the results of these investigations have been reported in preliminary form (17, 21, 22, 29).

The results of blood electrolyte studies carried out on a series of heart-lung preparations with and without the administration of a cardiac glycoside are reported in this paper.

METHODS. The heart-lung preparations were set up as described by Peters and Visscher (23). External diastolic volume, arterial pressure, and oxygen consumption were recorded continuously in most of the preparations. External output and heart rates were recorded at frequent intervals throughout the experiment.

Blood samples were taken at the beginning and at intervals for the duration of the preparation, and the serum analyzed for potassium by a modification of the silver cobaltinitrite method of Breh and Gaebler (24). Whole blood potassium, glucose, cell volume, and serum sodium, calcium, and chloride analyses were also carried out in part of the experiments.

The experiments have been controlled and amplified by additional experiments with the completely isolated heart-oxygenator preparation (25) and with completely isolated, blood perfused, artificially respiring lungs.

In a few experiments serum potassium analyses of samples from blood kept in a tonometer in the heart-lung constant temperature bath were carried out at identical intervals along with the similar analyses of the circulating heart-lung blood.

Varying doses of a digitalis glycoside (usually one of the pure glycosides of *Digitalis lanata*, Lanatoside A, B or C) were given to the majority of the preparations studied; the remainder, receiving no drug, served as controls.

The heart-lung or completely isolated heart fails progressively from the outset. This failure is characterized by a decrease in the mechanical efficiency of the heart, so that as the heart fails it continually liberates greater amounts of energy to perform the same amount of work. If such a heart is kept at constant external diastolic volume by continuously reducing the venous return, the external work of the preparation progressively decreases while the total energy liberation (oxygen consumption) remains constant as predicted by Starling's diastolic volume law (25, 26). If a suitable dose of a digitalis glycoside is administered this process of progressive failure is arrested or reversed and an increase in the external mechanical efficiency of the preparation occurs (23, 25). Depending upon the dose of glycoside, the period of increased efficiency is maintained for a time and then the heart again fails, either maintaining its normal rhythm (therapeutic dose) like an untreated heart, or developing sudden typical digitalis

irregularities with rapid failure (toxic dose). In the experiments reported here toxic and therapeutic doses of digitalis are defined on this basis (25): A toxic dose of a digitalis glycoside is one which produces cardiac arrhythmias in the heart of the heart-lung preparation. A therapeutic dose is one which will increase the external mechanical efficiency of the heart-lung heart without the production of cardiac arrhythmias within a period of 150 minutes after administration of the drug.

RESULTS. Serum potassium concentration changes have been followed in 75 heart-lung and isolated heart preparations and in a number of control experiments. In some of these experiments whole blood potassium, glucose, cell
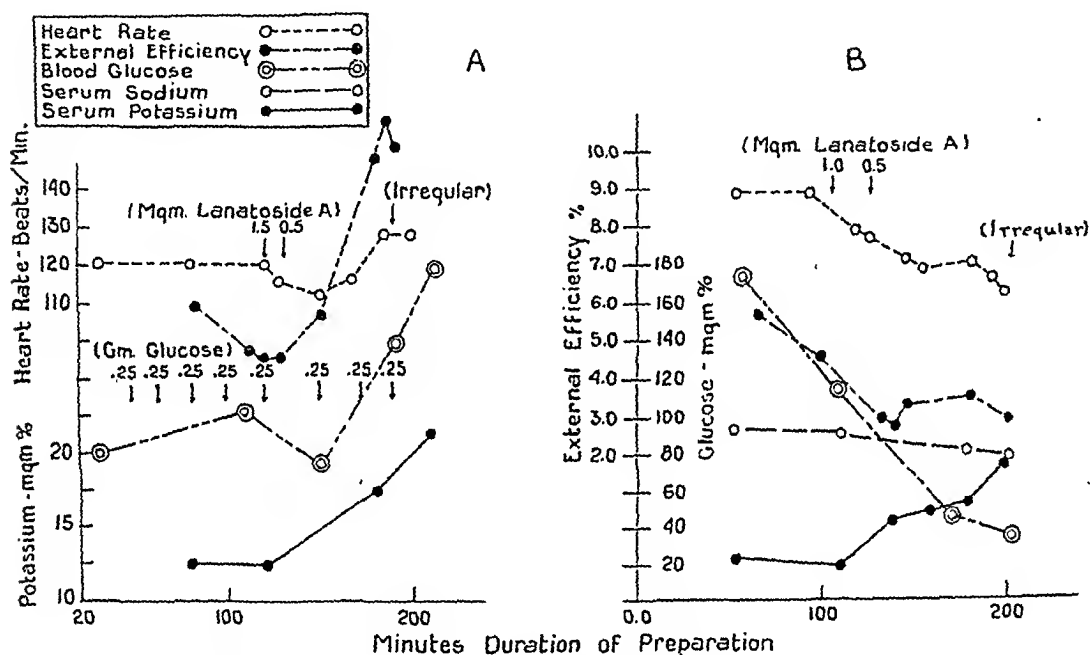
Fig. 1. Heart-lung preparations which received "toxic doses" of Lanatoside A

	A	B
Dog weight	9.10 kgm.	10.5 kgm.
Final lung weight	235 grams	215 grams
Final ventricular weight	102 grams	98 grams
Final blood volume	1535 cc.	1315 cc.

volume, and serum sodium, calcium, and chloride analyses have also been carried out.

The results of the analyses and the external mechanical efficiency of the preparation are plotted against the duration of the preparation for typical experiments in figures 1 and 2.

Control experiments. In experiments without digitalis, whole blood and serum potassium usually increase very slightly during the first two hours of the experiment and then increase at a somewhat faster rate for the duration of the preparation. This terminal increase in the rate of serum potassium increase is usually associated with severe pulmonary edema which is the probable causative factor.

Cell volume increases slowly throughout the experiment due to hemoconcentration resulting from the progressive formation of cardiac and pulmonary edema. Serum sodium, chloride, and calcium usually remain essentially constant, while blood glucose falls progressively throughout the experiment. The external mechanical efficiency of these preparations decreases continuously.

Digitalis glycoside experiments. Typical observations are shown in figures 1 and 2. In these experiments the control periods before administration of the drug were similar in all respects to the control experiments. When compared
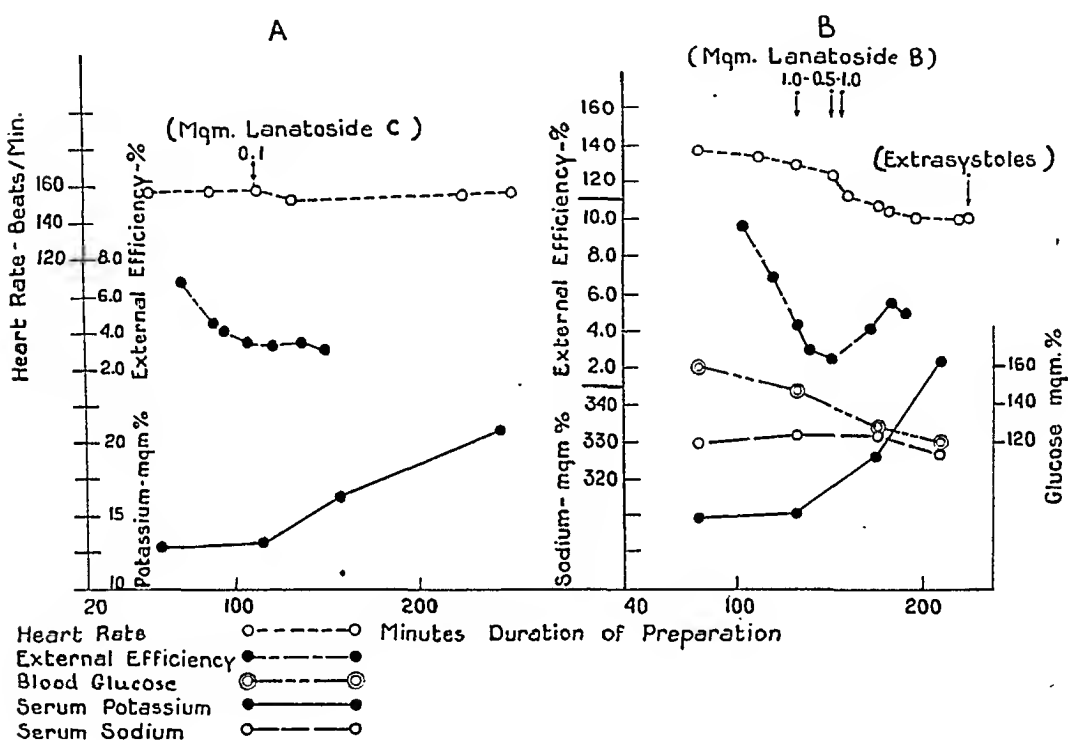
Fig. 2. Heart-lung preparations which received: A, a "therapeutic dose" of Lanatoside C; B, a "toxic dose" of Lanatoside B.

	A		B	
Dog weight..	12.3	kgm.	7.95	kgm.
Final lung weight............................	182	grams	440	grams
Final ventricular weight......................	88	grams	76	grams
Final blood volume..........................	1382	cc.	1390	cc.

to the control experiments administration of the digitalis glycosides had very little effect on the changes in cell volume, blood glucose, serum chloride, or calcium. The serum sodium showed no change except when very large doses were used when it usually declined. In contrast to these negative findings the blood potassium showed significant and quite uniform changes.

In the experiments receiving toxic doses of a digitalis glycoside there was a marked increase in whole blood and serum potassium. Preparations receiving therapeutic doses usually show a slight increase over control experiments in the

rate of increase of serum potassium. Therapeutic or toxic doses of these glycosides arrested or reversed the progressive fall in mechanical efficiency for periods of varying duration. Nearly all these preparations were kept at constant external ventricular diastolic volume for approximately the first two hours of the experiment. Therefore in untreated preparations the work output of the heart was continuously decreasing throughout this period. In treated preparations this same decreasing work output occurred during the control period but therapeutic or toxic doses of a digitalis glycoside were followed by a substantial increase in the work output of the heart. This fact may possibly have a bearing on the potassium exchanges which occur, since Fenn (31) has shown that potassium lost from striated muscle during contraction varies directly with the amount of tension developed. The heart rate usually remained essentially constant throughout the untreated and therapeutic dose experiments. The arrhythmias and consequent rate changes which follow toxic doses of digitalis glycosides usually do not occur until relatively late in the experiment.

The relative total cardiac and blood content of sodium, chloride, calcium, water, and potassium are such that only potassium exchanges can be satisfactorily analyzed by means of blood concentration changes. Sodium, chloride, and water exchanges can be more satisfactorily studied by means of direct tissue analyses (28). Therefore only the potassium exchanges which occurred will be considered in more detail.

The following calculations based on the serum and whole blood potassium analyses have been carried out in an attempt to obtain information concerning the following problems: 1. Do therapeutic as well as toxic doses of digitalis glycosides cause an increase in blood potassium? 2. Is the magnitude of the serum or blood potassium increase proportional to the dose of the digitalis glycoside? 3. Is the blood potassium increase a result of the toxic actions of these drugs or is it related to their cardiac efficiency increasing effect? 4. What are the absolute and relative potassium mobilizing, efficiency increasing, and toxic powers of the individual glycosides studied? 5. Does the increased blood potassium originate from the heart, the lungs, or both?

Any attempt to approach a quantitative comparison of potassium exchanges based on blood potassium concentration changes in this type of experiment must allow for such variables as blood volume, duration of experiment, and the size of heart and lungs. To avoid this difficulty total blood potassium increases were calculated by multiplying the increase in the blood concentration of potassium by the total blood volume. Blood potassium increases were then expressed as rates of blood potassium increase in terms of milligrams of potassium per gram of initial ventricular weight per hour. The initial ventricular weight was calculated from the final ventricular weight by subtracting the weight of the edema fluid as calculated from chloride analyses or from the lung edema-cardiac edema correlation curve (27). Since there is a good positive correlation between lung weight and heart weight, expressing exchanges on the basis of heart weight also avoids most of the potassium exchange variation which could be expected from variation in lung weight. Most of the potassium exchanges have been calculated on

the basis of serum potassium concentration changes, since it is difficult to correct whole blood potassium concentration changes for changes in cell volume resulting from hemoconcentration. Serum potassium exchanges undoubtedly include some potassium lost from the red cells, since serum potassium concentration usually increases slightly in samples of stirred blood. This movement of potassium from the red cell to the serum is apparently small, however, and not affected by the digitalis concentrations used in these experiments. When serum potassium concentration is increasing relatively rapidly, the increase in whole blood potassium concentration tends to lag, due to the delay in the distribution of the added potassium across the red cell membrane (figs. 5 and 6).

Only the potassium exchanges which occur during the period of increased efficiency due to the glycoside will be considered in detail. Exchanges occurring in this period are probably of the greatest significance, since this period is relatively early in the life of the preparation when the complicating factors of cardiac and lung edema are at a minimum. The importance of these observations lies in the following: If the serum potassium increases accompany the therapeutic action of digitalis it is possible that mobilization of tissue potassium may be related to the therapeutic action of the drug.

In an attempt to determine the effect of therapeutic doses of digitalis glycosides on the serum potassium, potassium exchanges have been calculated for the ten experiments which received therapeutic doses of Lanatoside C or Ouabain. The results of these calculations with the pertinent data for each experiment are given in table 1. As a control for these results, the same data are given for comparable periods from 12 experiments which received no drug (table 2). The average values and range of variation of the potassium exchanges of these experiments and also from 21 experiments which received toxic doses of a digitalis glycoside are given in table 3. These tables indicate that therapeutic glycoside doses cause a small but apparently significant increase in the rate of serum potassium increase over the control preparations. The approximate probable error of a blood serum potassium analysis is 0.25 mgm per cent (2, 24). Ventricular tissue analyses of therapeutic dose hearts also indicate a loss of potassium from these hearts (28). It should be pointed out that the differences between the rates of blood potassium increase of the control and therapeutic dose experiments would be more evident if longer periods of drug action were chosen. The period of duration of the drug action studied was limited to the time during which the external mechanical efficiency was increased over the efficiency which was present before the drug action.

Toxic doses of a digitalis glycoside cause a very large increase (15-fold) in the rate of serum potassium increase even during the efficiency increase period of the drug's action when as yet no major toxic effects are detectable.

The averages and extremes for the calculated potassium exchanges during the efficiency increase period and other pertinent data for the experiments with Lanatosides A, B and C are given in table 4, along with similar data from comparable periods of control experiments and from the control periods of the drug experiments. The data included in table 4 show conclusively that the pure

glycosides of *Digitalis lanata* cause a highly significant increase in the blood potassium content of the heart-lung preparation.

The rate of blood potassium gain has been plotted against the total dose of the drug (expressed as milligrams of drug per 100 grams of initial ventricular weight) for each experiment. The results for the experiments with Lanatoside A are shown in figure 3. The correlation coefficients between dose and rate of blood potassium increase has been calculated[5] for each of the Lanatosides with the following results: Lanatoside A, 0.873 ± 0.163; Lanatoside B, 0.853 ± 0.151; Lanatoside C, 0.959 ± 0.100. These correlation coefficients are statis-

TABLE 1

Effects of cardiac glycosides on the heart-lung preparation: blood potassium increase during the period of increased efficiency produced by therapeutic doses of cardiac glycosides in relation to the amount of drug and the efficiency increase

EXPERIMENT	DRUG	GM. CALC. INIT. VENTRICLE WEIGHT	DOSE MGM./100 GM. INIT. VENTRICLE	BLOOD VOL.	DOSE MGM./LITER BLOOD	TIME FROM START OF PREPARATION Drug given	TIME FROM START OF PREPARATION Sample during eff. incr.	PERIOD	INCR. BLOOD K	TOTAL INCR. BLOOD K	RATE BLOOD K INCR. MGM./ GM. OF VENTRICLE/HR.	EFFIC. WHEN DRUG GIVEN	INCR. EFFIC.	INITIAL SERUM K
				cc.				min.	mgm. %	mgm.		%	%	mgm. %
M-35	Lanat. C	63	0.159	1079	0.093	115	143	28	0.7	7.56	0.270	6.52	0.48	17.8
M-36	Lanat. C	74	0.135	1064	0.094	173	200	27	0.7	7.45	0.224		+	15.7
M-38	Lanat. C	45	0.111	984	0.051	76	105	29	1.6	18.7	0.740	5.65	0.25	12.0
M-40	Lanat. C	74	0.135	1114	0.090	112	158	46	1.8	20.0	0.363	3.33	0.17	14.5
M-46	Lanat. C	58	0.172	1287	0.078	42	80	38	0.5	6.43	0.178	3.40	0.38	15.4
M-47	Lanat. C	79	0.051	1127	0.036	89	107	18	0.0	0.0	0.0	4.88	0.92	10.4
M-97	Ouabain	67	0.015	1310	0.008	60	88	28	0.2	2.65	0.084	4.78	0.20	16.7
M-98	Ouabain	52	0.017	1290	0.007	57	87	30	0.6	7.70	0.298	7.50	?	15.8
M-100	Ouabain	57	0.044	1500	0.017	65	95	30	0.5	7.50	0.264	6.91	1.12	14.3
M-101	Ouabain	124	0.040	1300	0.038	58	90	32	0.0	0.0	0.0		+	16.4
Average		69	Lan. C 0.127 Ouabain 0.029	1200	Lan. C 0.074 Ouabain 0.018	85	115	31	0.66	7.80	0.242	5.37	0.50	14.9

tically highly significant, indicating a positive correlation between the rate of blood potassium increase and the dose of each glycoside. The slopes of the correlation lines for individual glycosides are significantly different, showing that each of the glycosides studied has a characteristic potency in producing an increase in the serum potassium of the heart-lung preparation. The correlation coefficients obtained when the dose of the drug was expressed in terms of blood

[5] The correlation coefficients were calculated as described by Fisher (33) without the use of Sheppard's adjustment. The calculated standard deviations were corrected for the size of the sample.

concentration were slightly smaller in each case, an indication that in the heart-lung preparation the effects of these glycosides are determined more by by the amount of drug per unit of tissue than by the concentration of the drug in the perfusion fluid (25). However, the difference between the correlation coefficients in the individual case was not statistically significant.

TABLE 2

Heart-lung preparations

Control experiments (no drug)

Comparable control periods for the efficiency increase periods of the therapeutic dose experiments. (50 min. period from 80 to 130 min. after the start of the preparation).

EXPERIMENT	GM. CALC. INIT. VENTRICLE WEIGHT	BLOOD VOL.	INCR. BLOOD K	TOTAL INCR. BLOOD K	RATE BLOOD K INCR. MGM./GM. OF VENTRICLE/HR.	EFFIC. START OF PERIOD	EFFIC. END OF PERIOD	INITIAL SERUM K
		cc.	*mgm.%*	*mgm.*		*%*	*%*	*mgm.%*
M-33	73	1306	−0.1	−1.30	−0.025			14.8
M-34	63	1132	0.3	3.40	0.065	6.00	3.45	18.4
M-39	50	1246	0.6	7.47	0.179	8.17	5.70	14.6
M-43	55	1136	0.1	1.14	0.025	4.65	4.25	12.8
M-58	56	1150	0.1	1.15	0.025			16.2
M-59	53	1172	−0.3	−3.52	−0.080			17.3
M-60	55	1104	0.5	5.52	0.120			15.7
M-62	63	1318	0.0	0.0	0.0			12.8
M-70	102	1200	−0.3	−3.60	−0.042			13.1
M-72	127	1200	0.0	0.0	0.0			14.4
B-2	67	1200	−0.2	−2.40	−0.042			12.5
B-3	83	1171	0.2	2.35	0.034			17.5
Average....	70	1200	0.07	0.85	0.041	6.27	4.50	15.0

TABLE 3

Average serum potassium changes during the period of increased cardiac efficiency produced by cardiac glycosides and in comparable control periods in heart-lung preparations

	NUMBER OF EXPERIMENTS	MGM. PER 100 CC.	SERUM POTASSIUM GAIN: MGM./GM. OF VENTRICLE/HOUR
No drug....................	12	0.07 (−0.3–0.6)	0.041 (−0.080–0.179)
Subminimal dose................	2	0.4 (0.3–0.5)	0.092 (0.074–0.190)
Therapeutic dose................	10	0.66 (0.0–1.8)	0.242 (0.0 −0.744)
Toxic dose....................	21	3.2 (1.5–9.5)	0.600 (0.285–1.12)

The available data do not allow a direct answer to the question of whether the serum potassium increases are related to the toxic, the therapeutic, or both types of digitalis action in the heart-lung preparation. Very probably the possible effects of the observed serum potassium increase on the function of the heart and lungs are mainly related to the rate of or total loss of potassium from the tissues or to local concentration changes rather than to the circulating serum

potassium concentration levels. This concept is supported by the fact that little or no correlation was found between the serum potassium concentration levels and the therapeutic or toxic actions of these drugs and also by experiments in which the serum potassium concentration was increased by injection of an isotonic solution of potassium chloride (29). In order to compare the calculated serum potassium exchanges with the toxic and therapeutic actions of these drugs

TABLE 4

Effects of cardiac glycosides on the heart-lung preparation: blood potassium increase during the period of increased efficiency produced by cardiac glycosides in relation to dose and efficiency increases*

NO.	DRUG	EDEMA CORRECTED VENTR. WEIGHT	TOTAL DOSE MGM./100 GM. VENTR.	BLOOD VOL.	TOTAL DOSE MGM./LITER BLOOD	TIME FROM START OF PREPARATION Drug given	TIME FROM START OF PREPARATION Sample during eff. incr.	PERIOD OF DRUG ACTION	INCR. BLOOD K	TOTAL INCR. BLOOD K	RATE BLOOD K INCR. MGM./GM. OF VENTR./HR.	EFFIC. WHEN DRUG GIVEN	INCR. IN EFFIC.	INITIAL SERUM K
		gm.		cc.				min.	mgm. %	mgm.		%	%	mgm. %
14	Control periods of drug Experiments	(55-122) 75	0.0	(1185-1440) 1290	0.0	(15-118) 63	(72-145) 107	(24-63) 45	(-0.5-1.2) 0.17	(-6.4-14.4) 2.07	(-0.096-0.261) 0.041	(4.13-16.50) 6.21	(-0.49-7.50) -2.68	(11.3-17.3) 13.8
12	Comparable periods of control† Experiments	(50-127) 70	0.0	(1104-1318) 1200	0.0	80	130	50	(-0.3-0.6) 0.07	(-3.6-7.5) 0.85	(-0.08-0.18) 0.041	(4.65-8.17) 6.27	(-0.40-2.51) -1.77	(12.5-18.4) 15.0
12	Comparable periods of control‡ Experiments	70	0.0	(1100-1300) 1180	0.0	125	190	65	(0.0-1.8) 0.69	(0.0-21.8) 7.92	(0.0-0.35) 0.122	(4.8-12.2) 6.92	(-2.3-6.0) -3.30	(12.5-17.5) 14.7
9	Lanatoside A Experiments	(46-126) 74	(0.86-4.31) 2.28	(900-1420) 1294	(0.40-2.35) 1.34	(63-200) 120	(90-270) 180	(27-123) 60	(1.5-6.0) 3.71	(18.7-70.3) 44.40	(0.24-1.12) 0.647	(0.71-6.50) 4.08	(0.0-6.37) 2.05	(12.3-16.7) 13.5
11	Lanatoside B Experiments	(50-122) 89	(0.74-6.47) 3.58	(900-1440) 1201	(0.63-6.12) 2.61	(43-134) 78	(83-184) 126	(31-78) 48	(1.0-9.5) 3.07	(9.3-85.5) 35.10	(0.07-1.12) 0.527	(2.50-6.30) 3.95	(0.0-3.92) 1.24	(11.1-15.6) 14.4
10	Lanatoside C Experiments	(45-102) 70	(0.05-1.01) 0.242	(984-1287) 1073	(0.025-0.91) 0.183	(42-115) 86	(80-168) 127	(21-79) 41	(0.0-3.8) 1.29	(0.0-43.2) 13.2	(0.0-1.21) 0.385	(0.30-6.50) 4.24	(0.25-5.63) 1.24	(10.4-17.8) 14.2

* Bracketed figures extreme values, unbracketed figures average values. † Comparable control periods for Lanatoside B and C experiments. ‡ Comparable control periods for Lanatoside A experiments.

it is necessary to devise some means of quantitating these toxic and therapeutic actions.

The therapeutic activity of the drugs studied in these experiments consists of their ability to produce an increase in the external efficiency of the heart-lung preparation. This ability may be measured for any of the drugs used by determining the amount of efficiency increase per unit of the drug or by determining the minimum dose of the drug which will arrest or reverse the decrease in ex-

ternal efficiency of the preparation. As might be expected from the variations in degree of failure, etc., in different preparations, the amount of external efficiency increase per unit dose of a digitalis glycoside varies considerably from experiment to experiment. The minimum efficiency increasing dose however is considerably more constant and has been adopted by Moe and Visscher (25) as a means of measuring the relative therapeutic activities of these drugs.

The toxic digitalis action manifested in these experiments consists of the production of various types of cardiac irregularities (various types of block, auricular or ventricular asystole, aberrant rhythms and extrasystoles, ventricular systolic arrest, and atrial or ventricular fibrillation). The most convenient measure of the toxicity of the dose of a glycoside used for any experiment is the duration of drug action before the onset of cardiac irregularities.
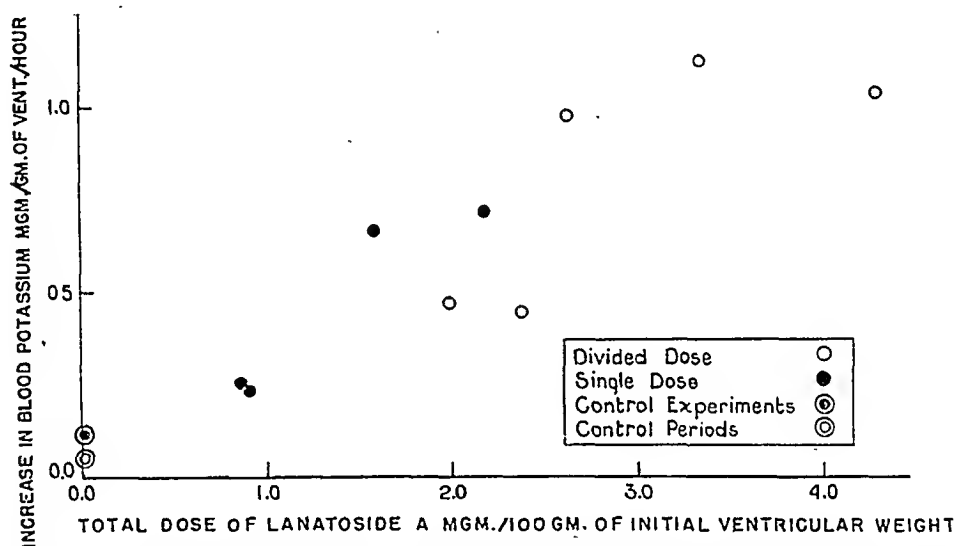
Fig. 3. Heart-lung preparations. Relationship of the total dose of Lanatoside A to the blood potassium increase during the period of increased external mechanical efficiency produced by the glycoside. (All doses "toxic," average duration of glycoside action: 59 minutes.)

If the serum potassium increases resulting from digitalis action were directly related to the irregularity producing action of these drugs, it might be expected that a correlation would exist between the time for the onset of these irregularities and the rate of serum potassium increase during the latent period of irregularity production. This possibility has been tested by plotting the duration of drug action before the onset of cardiac irregularities against the rate of serum potassium increase during this period. The correlation coefficient, calculated from the 20 experiments in which irregularities occurred after administration of one of the Lanatosides, was -0.303 ± 0.224. This correlation coefficient would occur in approximately one out of every five 20-sample trials because of errors in random sampling alone. Apparently little if any correlation exists between these two actions of the digitalis glycosides studied.

If the serum potassium increases are related to the efficiency increasing action of the digitalis glycosides a correlation might be expected to exist between the magnitude of the efficiency increase and the rate of serum potassium increase during the efficiency increase period. The increase of external efficiency has been plotted against the rate of serum potassium increase for both the toxic and therapeutic dose Lanatoside experiments (fig. 4). Reference to figure 4 shows that the increases in efficiency and increases in potassium liberation are concomitant phenomena. The correlation coefficient calculated from these data is 0.790 ± 0.123, indicating that a statistically significant positive correlation exists between these two actions of the digitalis glycosides studied.

In these heart-lung preparations it has been found that the product of the initial dose of any one digitalis glycoside and the duration of drug action before
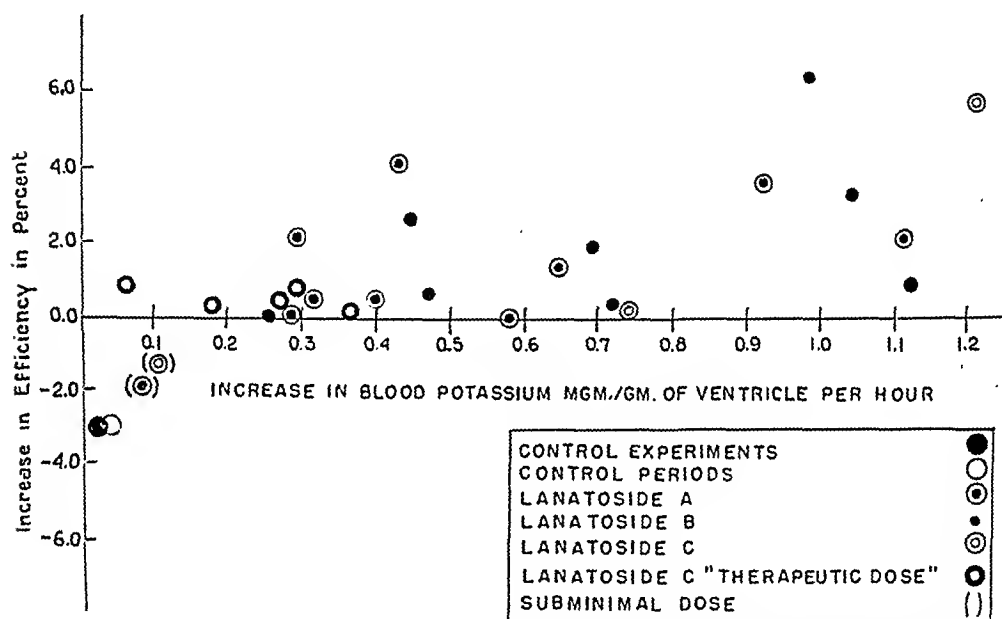
Fig. 4. Heart-lung preparations. Relationship of the blood potassium increase during the period of increased external efficiency produced by the glycoside to the increase in external mechanical efficiency of the heart.

the onset of cardiac irregularities is approximately constant. Each glycoside studied had a characteristic "dose-irregularity constant" which is an inverse measure of the drug's toxicity for the heart lung preparation.

The potassium mobilizing power of each glycoside has been calculated by dividing the average rate of potassium increase in the experiments carried out with each glycoside by the average dose of the glycoside. The relative potencies of the different glycosides studied in producing various toxic and therapeutic effects have been compared (table 5). Table 5 indicates that in the heart-lung preparation the relative toxicities of these glycosides correspond poorly with their relative efficiency increasing powers, and that their relative potassium mobilizing power corresponds somewhat more closely to their relative efficiency increasing activity than to their relative toxicities.

The evidence presented is of an indirect nature but it uniformly indicates that the liberation of potassium from the tissues in the heart-lung preparation by digitalis is more closely related to the therapeutic efficiency increasing effect than to the toxic irregularity producing action of the glycosides studied.

The problem as to the source of the increased blood potassium resulting from digitalis action in the heart-lung preparation has been attacked from several different angles and the results allow definite conclusions. Ventricular musculature analyses (28) clearly show that at least part of the potassium is liberated from the heart muscle. Additional proof of this obtained from experiments on the completely isolated heart preparation. In this preparation digitalis also causes an increase in blood potassium (fig. 5). The only possible source for the increased blood potassium in this type experiment is the heart muscle.

TABLE 5

Relative toxic and therapeutic potencies of cardiac glycosides

	ABSOLUTE VALUES				RELATIVE VALUES			
	Lanatosides			K stroph.	Lanatosides			K stroph.
	A	B	C		A	B	C	
Minimum lethal dose (mgm. per kgm.) Cat...............	0.32	0.40	0.23		1.20	1.00	1.33	
Dose-irregularity constant* Heart-lung....'...........	0.88	1.06	0.19	0.077	1.21	1.00	5.57	13.7
Minimum efficiency dose† Heart-lung.........'':......	0.857	1.750	0.097	0.048	2.03	1.00	17.9	36.4
Potassium mobilizing power‡ Heart-lung.................	0.283	0.147	1.58	3.66	1.92	1.00	10.8	24.9

* (Dose X minutes drug action before irregularities)/100.

† Milligram of drug per 100 grams of edema corrected ventricular weight.

‡ Milligram of serum potassium increase per gram of ventricle per hour per milligram of drug. Cat lethal dose data taken from Kaplan and Visscher (1940).

That part of the increase in blood potassium resulting from digitalis action on the heart-lung preparation originated from the lungs can be deduced by comparing the average loss of ventricular potassium determined by tissue analyses (28) with the much greater average total increment in blood potassium. Experiments with completely isolated, artificially respiring, blood perfused lungs show that in these preparations digitalis glycosides can cause a large increase in blood potassium content (fig. 6) which must originate from the lungs.

In a number of experiments the initial lung potassium level has been determined by removal of a lobe of the lung for analysis at the start of the preparation. A knowledge of the total blood potassium increment coupled with cardiac and lung analyses at the end of the preparation allow a calculation of the approximate potassium exchanges between the blood, the heart and the lungs which occurred during the experiment. Calculations based on this type of experiment indicate that in control experiments practically all of the relatively small blood potassium increment originates from the lungs.
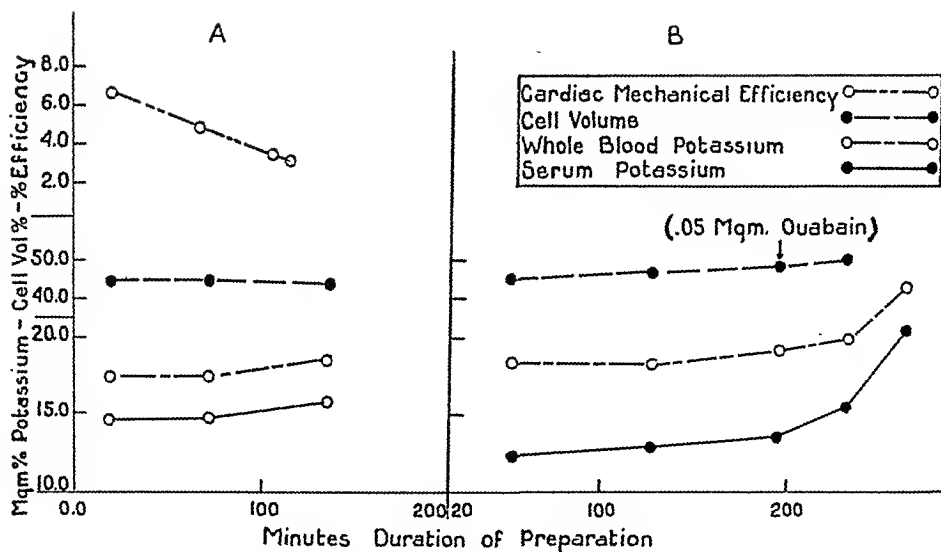
Fig. 5. Completely isolated heart preparations which received: A, no drug; B, a
"therapeutic dose" of Ouabain

	A	B
Dog weight	9.10 kgm.	23.0 kgm.
Final ventricular weight	68 grams	213 grams
Final blood volume	1400 cc.	1400 cc.
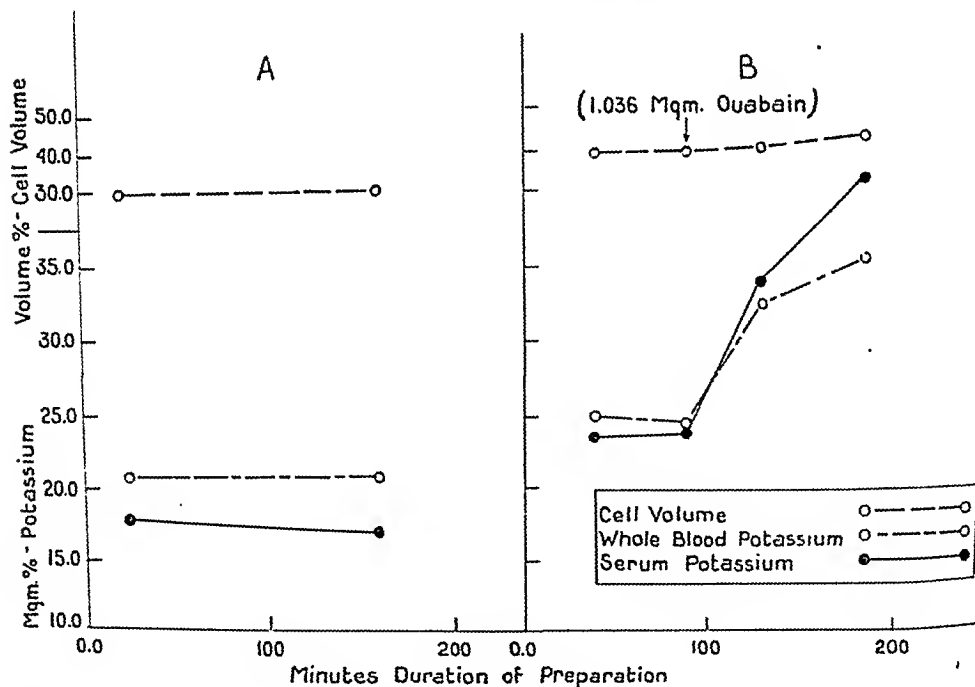
Fig. 6. Completely isolated perfused lung preparations which received: A, no drug; B, a
"toxic dose" of Ouabain

	A	B
Dog weight	16.8 kgm.	27.0 kgm.
Final lung weight	185 grams	710 grams
Final blood volume	1200 cc.	1230 cc.

In experiments receiving toxic doses of digitalis glycosides both the heart and the lungs lose considerable amounts of potassium, the loss from the lungs frequently accounting for more than half of the total blood potassium increment. These calculations are based on the assumptions of a normal lung weight to body weight ratio and the homogeneity of the lung tissue potassium content, so that they are of little value as a means of calculating quantitative exchanges.

DISCUSSION. The similarity that exists between the water, potassium, and sodium content of voluntary and cardiac muscle cells (30) lends considerable support to the view that the electrolyte and water exchanges which result from voluntary muscle activity may also result from cardiac muscle activity. This concept gains indirect additional support from the present studies on the effects of cardiokinetic glycosides on the electrolyte metabolism of the heart-lung preparation.

The digitalis-electrolyte studies carried out on the heart-lung preparation confirm in most respects previous investigations carried out with other type preparations. The lack of sensitivity of the methods used in the earlier studies, however, precluded the determination of the relatively small potassium exchanges which might be expected to result from smaller doses of digitalis. The present studies, although they do not offer incontrovertible proof, show that in all probability "therapeutic doses" of digitalis in the heart-lung preparation cause the same type of electrolyte and water exchanges as those which result from toxic doses of digitalis, the only essential difference being in the magnitude of these exchanges. The cardiac muscle cell electrolyte exchanges resulting from digitalis action are apparently almost identical with the voluntary muscle cell electrolyte exchanges resulting from contraction, namely, a loss of muscle cell potassium in exchange for an equivalent amount of sodium (28).

The cause of these cardiac muscle cell electrolyte exchanges is a matter of speculation. It has been conclusively demonstrated that digitalis has direct effect on the contractile power of cardiac musculature, increasing the amount of tension developed during a contraction, the capacity for work output and the fraction of the total energy liberated which is converted to work (mechanical efficiency). Fenn (31) has shown that the amount of potassium lost from voluntary muscle cells during activity varies directly with the amount of tension developed. It does not appear unreasonable to postulate that the potassium loss from cardiac muscle during digitalis action may result from increased cardiac activity (increased work output and tension developed), i.e., the therapeutic action of digitalis. Such a postulate based upon the correlations found between therapeutic digitalis effects (increased work output) and tissue potassium mobilization and the similarities between the electrolyte composition of cardiac and voluntary muscle appears to be one of the simpler, logical interpretations of the results obtained in this study.

The actual cause of the potassium loss from muscle cells during contraction remains, as yet, an unsolved problem. Evidence is accumulating (1) which indicates that potassium loss from muscle may be connected with the fundamental energy liberating processes of muscle contraction. That digitalis in

some manner effects these processes, and that potassium loss from the muscle may be connected with this action of digitalis does not seem unlikely.

The concept that potassium loss from cardiac muscle is purely a toxic manifestation of digitalis action as postulated by several investigators is not supported by these studies. Therapeutic digitalis effects as well as the toxic effects are accompanied by increased rates of serum potassium increase. Little if any correlation can be found between rates of serum potassium increase, total serum potassium increase, or serum potassium concentration and the toxic digitalis effects. Guttman and Cattell (32) have shown that increase of the potassium concentration at the surface of striated muscle cells will, under special circumstances, produce an increase in twitch tension similar to that produced by ouabain. They found that increased extracellular potassium did not cause the decreased efficiency of contraction (toxic effect) resulting from digitalis action.

The toxic and the therapeutic actions of a drug are in many instances gradations of the same fundamental actions. From this point of view, therapeutic doses of digitalis produce cardiac potassium exchanges which are in a sense comparable to those occurring in normal voluntary muscle contractions, while toxic doses of digitalis produce losses of cardiac potassium which are comparable to the potassium loss from voluntary muscle which occurs when the muscle is stimulated to exhaustion.

The actual cause of the toxic digitalis effects (arrhythmias) seen in these experiments has not been elucidated; however, these effects do not appear to be produced by changes in the distribution of potassium alone.

SUMMARY

The effects of digitalis glycosides on blood electrolyte levels have been studied in 75 heart-lung preparations and in a number of control experiments. In some of these experiments whole blood potassium, glucose, cell volume, and serum sodium, calcium, and chloride analyses have also been carried out. These analyses and calculations based upon these analyses lead to the following conclusions: 1. In an untreated control heart-lung preparation a variable degree of hemoconcentration, a tendency for a slow increase in serum potassium, and a progressive fall of blood glucose occur. Constant changes in serum, sodium, calcium, and chloride were not found. 2. Suitable doses of digitalis cause concomitant increases in external mechanical efficiency and blood and serum potassium along with a questionable decrease in serum sodium. Significant differences from the untreated preparations were not demonstrated in the other blood components studied. 3. Therapeutic doses of digitalis glycosides produce relatively small but apparently significant increases in serum potassium. 4. A positive correlation was demonstrated between the total dose of a digitalis glycoside and the rate of serum potassium increase both during the efficiency increase period and for the duration of the drug's action. 5. The increased external efficiency and increased rate of potassium liberation which occur after suitable doses of digitalis are concomitant phenomena but a simple relationship between these two actions of digitalis on the heart-lung preparation is not evi-

dent. 6. Little if any correlation was found between the time of onset of digitalis irregularities and the rate of serum potassium increase or the potassium concentration of circulating serum. 7. The relative potassium mobilizing powers of the glycosides studied correspond somewhat more closely to their relative therapeutic activities than to their relative toxicities for the heart-lung preparation. 8. An increase in blood and serum potassium results from the action of suitable doses of digitalis glycosides in both the completely isolated heart and the completely isolated lung preparations. 9. The increased blood potassium resulting from digitalis action on the heart-lung preparation originates from both the heart and lung tissue.

REFERENCES

(1) FENN, W. O. Physiol. Rev. **16:** 450, 1936; ibid. **20:** 377, 1940.

(2) WOOD, E. H., D. A. COLLINS AND G. K. MOE. This Journal **128:** 635, 1940.

(3).HOWELL, W. H. AND W. W. DUKE. This Journal **21:** 51, 1908.

(4) LEHNHARTZ, E. J. Physiol, **86:** 37P, 1936.

(5) KEHAR, N. D. AND E. V. McCOLLUM. This Journal **110:** 485, 1934.

(6) KEHAR, N. D. AND D. R. HOOKER. Ibid. **112:** 301, 1935.

(7) CALHOUN, J. A., G. E. CULLEN, G. CLARKE AND T. R. HARRISON. J. Clin. Investigation **9:** 393, 1930.

(8) STEWART, H. A. J. Exper. Med. **187:** 13, 1911.

(9) MANGUN, G. H. AND V. C. MYERS. Proc. Soc. Exper. Biol. and Med. **35:** 455, 1936–37.

(10) DECHERD, G., E. H. SCHWAB, G. HERRMANN AND W. O. BROWN. Proc. Soc. Exper. Biol. and Med. **33:** 521, 1935–36.

(11) MACKAY, E. M. AND H. C. BERGMAN. J. Clin. Investigation **11:** 497, 1932.

(12) SHELLEY, W. B. AND C. F. CODE. This Journal **133:** P444, 1941.

(13) DENNIS, J. AND R. M. MOORE. This Journal **123:** 443, 1938.

(14) SCUDDER, J., M. E. SMITH AND C. R. DREW. Ibid. **126:** 337, 1939.

(15) HERRMANN, G. AND P. ERHARD. Proc. Soc. Exper. Biol. and Med. **38:** 35, 1938.

(16) CALHOUN, J. A. AND T. R. HARRISON. J. Clin. Investigation **10:** 139, 1931.

(17) WOOD, E. H. AND G. K. MOE. This Journal **123:** 219, 1938.

(18) WEDD, A. M. J. Pharmacol. and Exper. Therap. **65:** 268, 1939.

(19) HAGEN, P. S. Ibid. **67:** 50 1939.

(20) BOYER, P. K. AND C. A. POINDEXTER. Am. Heart J. **20:** 586, 1940.

(21) WOOD, E. H. AND G. K. MOE. This Journal **129:** P499, 1940.

(22) MOE, G. K. AND E. H. WOOD. Ibid. **129:** P425, 1940.

(23) PETERS, H. C. AND M. B. VISSCHER. Am. Heart J. **11:** 273, 1936.

(24) WOOD, E. H. J. Lab. and Clin. Med. **27:** 960, 1942.

(25) MOE, G. K. AND M. B. VISSCHER. This Journal **125:** 461, 1939.

(26) MOE, G. K. AND M. B. VISSCHER. J. Pharmacol. and Exper. Therap. **64:** 65, 1938.

(27) WOOD, E. H. AND G. K. MOE. This Journal **136:** 506, 1942.

(28) WOOD, E. H. AND G. K. MOE. This Journal **136:** 515, 1942.

(29) WOOD, E. H. AND G. K. MOE. Fed. Proc. Pt. II, **1:** 95, 1942.

(30) WOOD, E. H. J. Biol. Chem. **143:** 165, 1942.

(31) FENN, W. O. This Journal **124:** 213, 1938.

(32) GUTTMAN, S. A. AND M. CATTELL. J. Pharmacol. and Exper. Therap. **68:** 267, 1940.

(33) FISHER, R. A. Statistical methods for research workers. Oliver and Boyd, Edinburgh, 1938.

DEPRESSION OF GASTRIC MOTILITY WITHOUT ELEVATION OF BODY TEMPERATURE FOLLOWING THE INJECTIONS OF PYROGENS[1]

H. NECHELES, P. DOMMERS, M. WEINER, WM. H. OLSON AND WM. RYCHEL

From the Department of Gastro-Intestinal Research of Michael Reese Hospital, Chicago

In the assay of substances which depress gastric motility authors have been careful to state whether or not such substances affected body temperature. It seems to be the consensus of opinion that slight elevations of body temperature do not affect gastric motility, while considerable elevations will depress it (2, 9, 10, 12). It seems to us that, in the light of our present findings, this opinion ought to be revised. Our present work was prompted by a number of observations on dogs in which subfebrile doses of pyrogenic substances were followed by more or less profound and prolonged inhibition of gastric motility (13).

The mechanism of gastric inhibition following elevation of body temperature is not known. Local diathermy of the stomach of dogs did not inhibit normal motility, although intragastric temperature was raised by 0.6° and 1°C. (1). On the other hand, exposure of dogs to high environmental temperatures was followed by inhibition (2, 3). Meyer and Carlson (2) produced fever of 39.4 to 40.6°C. in dogs by injections of sodium nucleate, peptone Witte and a vaccine of B. prodigiosus; absence of hunger contractions and gastric atony were observed. Temperature elevations of only 1 or 2 degrees above the normal had little or no effect. Inhibition of intestinal motility by pyrogenic substances has been reported by various authors, but body temperatures were not stated (4–6). Section of the vagus nerves abolished gastric inhibition following the injection of pyrogenic substances, although temperature reactions occurred (2). Various bacterial toxins did not affect strips of the dog's stomach (2). These observations point to a central mechanism for inhibition. On the other hand, the findings of Faber and others, on the occurrence of gastritis in infectious disease points to local toxic effects on the gastric mucosa (7).

In the human a slight elevation of body temperature may or may not depress gastric motility depending, apparently, not so much on the elevation of body temperature, but on the nature of the agent producing fever. Thus Beaumont reported among his observations on Alexis St. Martin that "in febrile diathesis or predisposition from whatever cause . . . the villous coat becomes sometimes red and dry, at other times pale and moist, and loses its smooth and healthy appearance" (8, p. 107). Carlson, reporting about his subject with a gastric fistula, Fred Vlcek, "he has had a few attacks of mild gastritis, in three cases associated with nose and throat colds with some temperature. During these attacks, the empty stomach remains somewhat atonic with complete absence of

---

the hunger contractions." Carlson has taken records on himself during two attacks of cold and tonsillitis with antrum infection; these "did not completely abolish the hunger contractions except when sufficiently severe to induce elevation of the body temperature to 101 or 102°F.," (38.3 or 38.9°C.), (9, p. 279). Meyer has reported more or less normal hunger contractions in most of his subjects suffering from various degrees of pulmonary tuberculosis. In these patients body temperature during the experiments varied between 37.2°C. and 38.3°C. (10). On the other hand, Rupp, who induced fever in himself and other subjects by injections of typhoid vaccine and sodium nucleate, found that when the temperature elevation reached 37.8 to 38.9°C. the strength or duration of the hunger contractions showed practically no deviation from the normal, but elevation of temperature between 40 and 40.5°C. usually rendered the empty stomach atonic (9, p. 286).

EXPERIMENTAL PROCEDURES. Normal healthy dogs were given stainless steel gastrostomy cannulas[2] in an aseptic operation and allowed to recover for two weeks. The animals were kept on a diet consisting of a commercial dog food containing meat, fat, rolled oats and vegetables, to which bone meal, brewers' yeast and cod liver oil were added. Once a week the animals received an ounce of dried ox blood with their diet. The dogs were healthy and of good appearance, most of them gaining weight. All animals had been in the laboratory for periods of six months to two years. The dogs were trained to lie on a padded table unrestrained, with their eyes covered. They were accustomed to intravenous injections and to the taking of rectal temperatures. Food but not water was withheld for 18 hours before each test performed once, and rarely twice, a week. A balloon was introduced into the stomach and connected to a manometer filled with an oil carbon tetrachloride mixture with a specific gravity of 1.5. The balloons were inflated gently with air to a water pressure of about 8 cm. Kymograph records of gastric motility were taken over periods of 4 to 8 hours. In order to obtain vigorous and continuous gastric motility, small doses of prostigmine[3] were injected subcutaneously 30 minutes before the beginning of the experiments, when the stomach cannula was opened and the gastric contents allowed to drain. The proper dose of prostigmine had to be found for each dog because too large a dose would produce vomiting and discomfort of the animal, while too small a dose would not be effective. By this method, continuous gastric motility of more than 5 hours' duration was obtained. Numerous controls of this stimulated gastric motility were taken and repeated at intervals on each of the nine dogs used. Rectal temperatures were taken at frequent intervals. All preparations used in the experiments reported below were administered intravenously. Before the injection of the pyrogenic substance a control of stimulated gastric motility was recorded in order to ascertain the presence of vigorous and continuous gastric activity. The room temperature at which the experiments were performed ranged between 70 and 75°F. At the lower range of room temperature the dogs were covered with blankets.

---

[2] Gold plated.
[3] Supplied by the Hoffman-La Roche Company.

The results of our experiments were computed in the following manner: The average height of contractions during the control period and following injection of a pyrogenic substance was determined and the effects of the pyrogens are expressed as percentage of the control values. In order to facilitate the reading of our table, + signifies 33 per cent depression, ++, 33 to 66 per cent, and +++, 66 to 100 per cent depression of stimulated gastric motility. Only such results are reported in which inhibition of 33 per cent and above occurred.

Usually, when a healthy dog is resting comfortably, his rectal temperature will drop for a few degrees Centigrade, but the contrary has been observed in a number of instances; thus a rise of 0.2°C. was not uncommon, and once a rise of 0.8°C. was observed. The s.c. injection of prostigmine did not seem to affect rectal temperature. The rectal temperature of the dog is not as constant as that of the human. However, in order to use rigid standards for non-pyrogenic effects of the substances employed in this study only such results are reported here, in which rectal temperatures of healthy dogs did not rise beyond 0.3°C. following the injection of these substances. Our procedure for the establishment of a dose of a pyrogenic substance which would not change the rectal temperature of a dog was to inject a test dose first, which usually evoked chills, high fever, vomiting, diarrhea, etc. In subsequent experiments this dose was gradually diminished, until no rise of rectal temperature occurred. In most of these latter experiments the animals remained comfortable, with the exception of a short chill and vomiting in some tests (see table 1). The results of experiments with fever reactions will be mentioned briefly only, since such observations have been described by others (v.s.). From a great number of experiments, in many of which rectal temperature rose only 0.4 to 1.0°C., a rise of temperature which many consider to be within normal limits for the dog and inconsequential in the evaluation of their results, we present here only such experiments (38 on 9 dogs), in which rectal temperature did not rise beyond 0.3°C. for a period of time of 2 to 4 hours after injection of the pyrogens.

The following substances were administered intravenously: Pentnucleotide,[4] thymus and yeast nucleic acid,[5] extracts prepared from various peptones,[5] B. coli vaccine and triple typhoid vaccine,[6] a crystalline preparation of bacterial pyrogen,[7] and ordinary tap water, brought to isotonicity by the addition of 0.9 per cent of NaCl. The nucleic acid preparations were alkalinized lightly with NaOH before injection.

RESULTS. Injections of pentnucleotide resulted in a 33 to 100 per cent inhibition of gastric motility in all 12 tests, with an average duration of 30 minutes. In one animal, complete suppression of gastric motility occurred with no change of rectal temperature, but the latter rose to 39°C. after normal gastric motility

[4] Supplied by Smith, Kline, and French. The manufacturers describe Pentnucleotide as a "mixture of the sodium salts of pentose nucleotides equivalent to 0.7 gram of pentose nucleotides per 10 cc."

[5] Prepared by Dr. M. Hanke, Dept. of Biochemistry, University of Chicago.

[6] Prepared by Dr. K. Howell and Mr. B. Halpern, from the Dept. of Bacteriology, Michael Reese Hospital.

[7] Containing 40 mgm. of solid material per cubic centimeter of solution.

had been resumed; the animal vomited at that time. In two other animals the period of depression of gastric motility was followed by considerable hypermotility of the stomach. In one dog the period of more or less complete depression was followed by a 60 minute period of contractions as high as during the control period, but of distinctly diminished rate.

TABLE 1

Depression of gastric motility by non-pyrogenetic doses of pyrogenic substances

SUBSTANCE	DOSES (INTRAVENOUS)		TOTAL NO. OF TESTS	NO. OF TESTS WITH DEPRESSION OF GASTRIC MOTILITY[1]				LATENT PERIOD IN MINUTES		DURATION OF DEPRESSION	
	Variation	Average		0	+ (33%)	++ (33-66%)	+++ (66-100%)	Variation	Average	Variation	Average
										minutes	*minutes*
Pentnucleotide	0.2–5.0 cc.	0.9 cc.	12			2	10[2]	½–4	1½	3–100	30
Nucleic acid, Thymus	10–100 mgm.	53 mgm.	7	7[3]							
Nucleic acid, Yeast	25–50 mgm.	37.5 mgm.	6				6[4]	1.0–3	1½	4–50	19
Peptone extract	5–150 mgm.	50 mgm.	4	4							
B. coli vaccine	12.5 millions		1		1[5]			10	10	40	40
Triple typhoid vaccine	25–150 millions	65 millions	8	3[6]	1	3[7]	1[8]	20–46	31	45–135	71
Crystalline bacterial pyrogen	0.1–0.001 cc. (4–0.04 mgm.)	0.023 cc. (0.092 mgm.)	6	2		1	3[9]	2–30	17	30–90	55
Tap water with 0.9% NaCl	10–50 cc.	26 cc.	4	1[10]		2	1	4–32	20	13–15	14

[1] Percent depression of stimulated gastric motility.

[2] In one animal temperature rose to 39°C. after cessation of inhibition and resumption of motility, and the dog vomited. In 2 animals depression was followed by considerable hypermotility. Another animal vomited after injection of 1 cc. of pentnucleotide. In another dog the rate of contractions fell from 4 per minute before injection of 1 cc. of pentnucleotide to 1 per 1–4 minutes. This occurred after depression of height of contractions had disappeared, and lasted for two hours.

[3] In one animal, 3 minutes after injection of thymus nucleic acid, considerable hypermotility, lasting 170 minutes, followed.

[4] One animal had a short chill during depression of motility, with rectal temperature of 38°C.

[5] At end of period dog panted and temperature rose from 37.3 to 38.5°C.; gastric tone and motility dropped +++ for 30 minutes.

[6] In one animal gastric motility increased considerably 3 hours after injection. In another animal chills and a diminished rate of contractions occurred (3 per minute in control, 1 per minute 65 minutes after injection, lasting one hour).

[7] One animal vomited at end of test.

[8] Short chills and sneezing; no changes in rectal temperatures.

[9] One animal vomited after depression was over and after vigorous motility had been resumed.

[10] One contraction in 3 minutes against 1 in 1 minute during control; average height of contractions in control 6 cm., after injection 2–14 cm.

Thymus nucleic acid had no depressant effect on gastric motility in all 7 experiments. In one animal, a considerable degree of hypermotility followed the injection, lasting for 170 minutes.

Yeast nucleic acid (fig. 1) had a profound depressing effect on gastric motility. One of these animals had a short slight chill during this period of depression but rectal temperature, taken during the chill, showed no variations from that of the control period. The chemical composition of the various nucleic acids is known to be different.

Peptone extracts had no effects on gastric motility and on body temperature. B. coli vaccine had a relatively slight depressant effect on gastric motility,

lasting for 40 minutes.   At the end of this period, the dog began to pant and
its rectal temperature rose from 37.3 before, to 38.5°C.   At the same time,
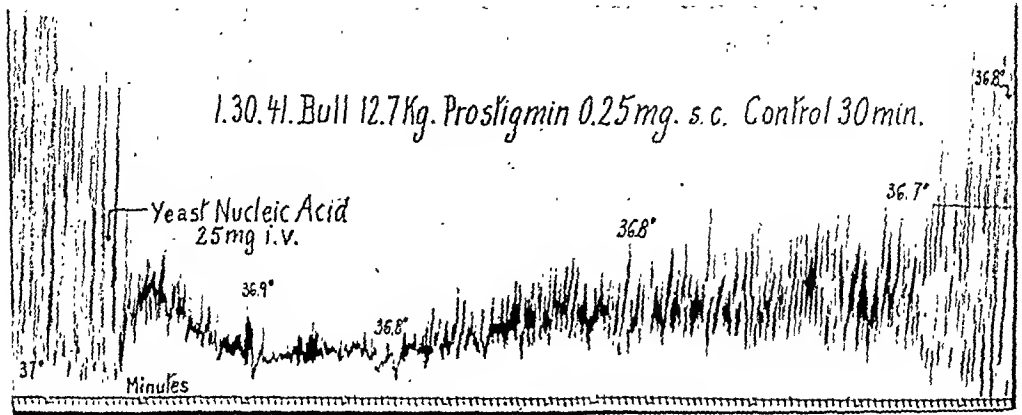
Fig. 1. Depression of stimulated gastric motility by yeast nucleic acid.   Note short
latent period.
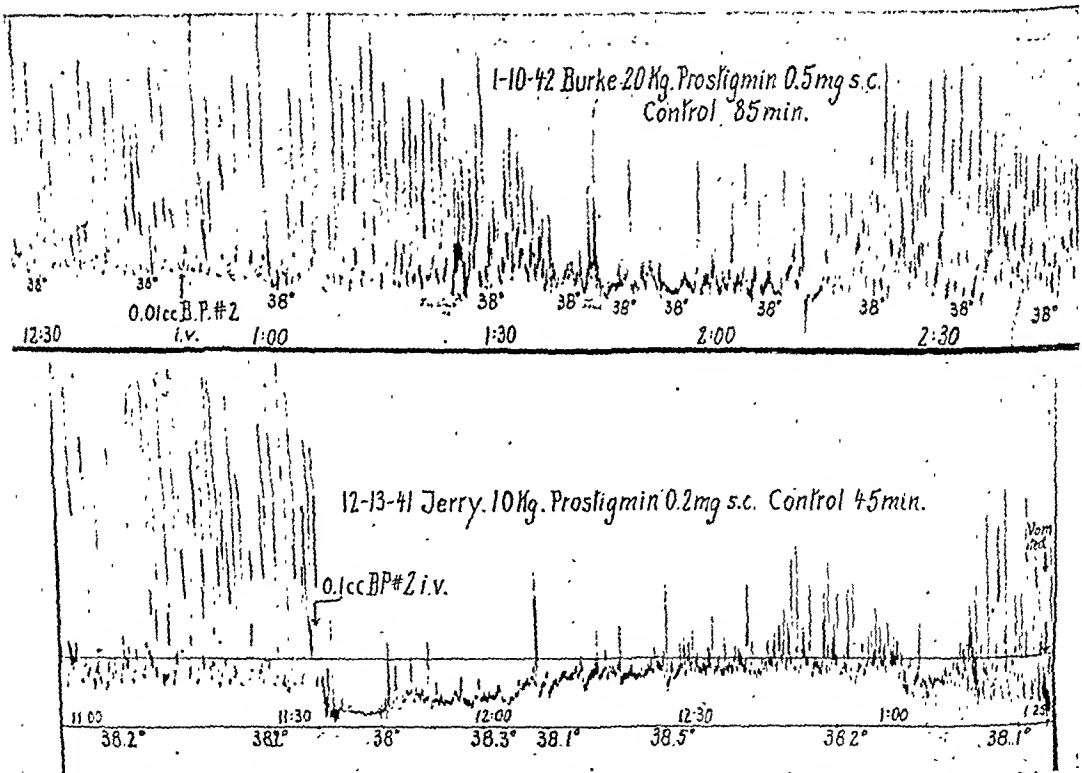
Fig. 2. Depression of stimulated gastric motility by crystalline bacterial pyrogen (B P
No. 2).   Note long latent period with smaller dose and short latent period with larger dose.

gastric tone and motility dropped precipitously for 30 minutes.   Panting per se
can raise the temperature of a dog, and the accompanying changes in the blood
may also affect the stomach.

Triple typhoid vaccine had no depressant effect in three experiments. In one of these, gastric motility increased considerably three hours after the injection, with no change in rectal temperature. In another test the rate but not the height of contractions decreased materially, but this result is counted as negative. In five other experiments with this vaccine, varying degrees of inhibition of gastric motility were obtained. In one of these experiments the animal vomited at the end of the test, but its body temperature was not elevated. In another one, short chills and sneezing occurred during the period of depression without a change in rectal temperature.

The crystalline preparation of pyrogen was ineffective in two animals and produced more or less profound depression of gastric motility in four other experiments with an average duration of 55 minutes (fig. 2).

Tap water, which usually contains pyrogens, produced depression of gastric motility in three experiments and was ineffective in one. In this latter experiment, the rate of contractions was one per minute during the control period, with an average height of 6 cm. Following the injection, the rate was one per three minutes, and the height varied between 2 and 14 cm. This may be considered inhibition because the rate of contractions was lowered, but we feel that the evaluation of this experiment is difficult, and therefore classified its result as negative.

DISCUSSION. A study of table 1 reveals that there was a more or less characteristic difference in the kind of depression produced by the various substances employed. The latent periods were relatively short in the case of pentnucleotide and yeast nucleic acid, and they were considerably longer with bacterial pyrogens. The periods of depression were considerably longer with typhoid vaccine and with the larger dose of crystalline pyrogen than with pentnucleotide, yeast nucleic acid, and tap water.

Some of the negative results reported in table 1 probably were due to insufficient doses and possibly also to individual variations in the susceptibility of the same or of different dogs to pyrogenic substances. Therefore, not all of the negative results can be taken as a proof that doses of pyrogens small enough not to affect rectal temperature will not have an effect on gastric motility. We feel that, in a number of the negative experiments, the dose of pyrogen administered was below the threshold necessary to produce gastric depression. In many experiments (not reported here) in which rectal temperature rose beyond 0.3°C. following the injection of substances employed in this study, pronounced inhibition of gastric motility resulted. In a great number of these experiments, body temperature did not rise beyond values considered by many within the normal range for the dog, i.e., 37.0 and 39°C. In others, fever of varying degree resulted. In most of these dogs, one or more of the following symptoms appeared: panting, chills, trembling, retching, vomiting, urination, defecation, and general discomfort. We feel that discomfort of the animal, possibly associated with increased activity of the adrenal medulla, may produce gastric inhibition. All of the substances used in this work were followed by profound depression of gastric motility, when given in larger doses. It is of

interest to note that a rise of rectal temperature could occur at the end of the period of inhibition, when gastric motility was on the increase (table 1).

Meyer (2) reported a few experiments in which rectal temperature did not rise beyond 0.3°C. following the injection of peptone Witte, but in all of these experiments vomiting, urination, defecation, dyspnea, restlessness, etc., were observed.

In a number of our experiments depression of gastric motility was not continuous; at times there appeared a release ("escape") of the inhibition and motility was resumed for more or less short periods of time. In some experiments the motility during those intervals was considerably greater than in the control period. This was followed by depression and, in a few experiments, the process was repeated a number of times. Wherever chills and vomiting occurred, they followed the larger doses of the substances employed. Chills were observed during motility as well as during depression. In a number of experiments gastric motility increased following injection of a pyrogenic substance.

Our results show that such small doses of pyrogenic substances which will not produce elevation of body temperature can produce more or less profound inhibition of gastric motility. This may be of interest to the physician who observes gastric disturbances in a great number of cases of colds, flu, antrum infection, etc., in which only subfebrile temperatures prevail. This may be due to minute amounts of pyrogenic substances released into the bloodstream in these infections.

Our results also indicate that we have to be extremely careful in assaying preparations which inhibit gastric motility, and that the mechanism of action of such preparations must be evaluated rigidly before claims for specificity are raised. Purified preparations of inhibitory substance from urine, which we have described first (see 11), are usually assayed for pyrogenic effects with the doses used to obtain depression of gastric functions. These doses do not produce temperature reactions in rabbits and dogs. Larger doses than necessary for obtaining distinct gastric depression will, however, produce pyrogenic reactions. It may be, therefore, that such and similar preparations are being used in subthreshold doses with regard to temperature reactions, but in sufficient doses to produce gastric inhibition. We have stressed this earlier (13) and recently Ivy's associates have agreed that their purified preparation from urine may contain traces of pyrogens (14). We do not want to assume here, however, that enterogastrone and the gastric inhibitory substance from urine owe their effects on the stomach solely to a content of pyrogen. We believe that they will be proven finally to be specific substances of a humoral character. We feel, however, that our results should be a warning for the rigid testing of such and similar biological materials, and for the elaboration of methods and experiments which will eliminate the presence of pyrogenic contents in these substances as possible factors in gastric depression.

Secretion experiments with the substances used in this work have shown conclusively that gastric secretion can also be inhibited without rise of body temperature.

SUMMARY

The effects of such small doses of pyrogenic substances that would not produce elevation of body temperature in the dog were tested on gastric motility stimulated by prostigmine. The following substances were used: Pentnucleotide, thymus and yeast nucleic acid, B. coli vaccine, triple typhoid vaccine, a crystalline preparation of a bacterial pyrogen, and tap water. In a great number of experiments, depression of gastric motility resulted with no change in rectal temperature and with no subjective signs in most of the animals. The relation of this finding to dyspepsia during colds and other infectious diseases in which no or only a slight elevation of body temperature occurs, is discussed. The importance of this finding in relation to biological preparations which depress gastric activity is pointed out because these preparations may contain subthreshold doses of pyrogens in regard to body temperature, but not in regard to gastric function.

REFERENCES

(1) Couta Silva, O. B. De. This Journal 87: 180, 1928.
(2) Meyer, J. and A. J. Carlson. This Journal 44: 222, 1917.
(3) Sleeth, C. K. and E. J. Van Liere. This Journal 118: 272, 1937.
(4) Ecker, E. E. and M. S. Biskind. Arch. Path. 7: 204, 1929.
(5) Galapeaux, E. A., H. F. Adler, R. L. Ferguson and R. D. Templeton. This Journal 123: 72, 1938.
(6) Adler, H. F., R. D. Templeton, R. L. Ferguson and E. A. Galapeaux. Am. J. Med. Science, 200: 514, 1940.
(7) Faber, K. Gastritis and its consequences. Oxford Medical Publications, 1935.
(8) Beaumont, W. Experiments and observations on the gastric juice and the physiology of digestion. Reprinted on the occasion of the 13th International Physiological Congress. Boston, Massachusetts, 1929.
(9) Carlson, A. J. The control of hunger in health and disease. Univ. of Chicago Press, 1916.
(10) Meyer, J. Arch. Int. Med. 22: 759, 1918.
(11) Necheles, H., M. E. Hanke and E. Fantl. Proc. Soc. Exper. Biol. and Med. 42: 618, 1939.
(12) Gray, J. S., C. U. Culmer, E. Wieczorowski and J. L. Atkinson. Proc. Soc. Exper. Biol. and Med. 43: 225, 1940.
(13) Necheles, H. Am. J. Digest Dis. 8: 382, 1941.
(14) Gray, J. S., E. Wieczorowski, J. A. Wells and S. C. Harris. Endocrinology 30: 129, 1942.

THE RÔLE OF THE VISCERA IN REGULATING THE TEMPERATURE OF THE BODY OF AN ANIMAL UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

N. A. FEDOROV AND E. I. SHUR

Of the Patho-Physiological Department of the Central Institute of Hematology and Blood Transfusion of the People's Commissariat of Health of the USSR, Moscow

The metabolic processes taking place in the cells of an organism are always accompanied by the production of heat. Numerous investigations of recent years have demonstrated the rôle of chemical regulation in maintaining constancy of temperature in warm-blooded animal organisms. Very little is known as yet, however, about the participation and comparative importance of the different viscera in this thermogenesis.

There are references in scientific literature to the rôle of the liver in the chemical regulation of temperature. R. Dubois showed that in the marmot the first rise in temperature after the period of hibernation occurred in the liver. Eck fistulae delayed the awakening of such animals. According to Cavazine, Hirsch, Müeller and Rolly, the liver is always warmer than the blood in the aorta; it may be assumed, however, that this is due to the accumulation in the liver of a large amount of venous blood, which is warmer than that in the left heart. Animals with the spinal cord sectioned at the thoracic level practically lose their capacity for thermo-regulation after the vagus has been cut. The same effect is obtained after cutting or ligating the hepatic artery. According to Plaut, the destruction of plexus hepaticus completely stops the "secondary chemical thermoregulation." Thermoregulation is lost in the curarized dog after denervation of the liver. No firmly established date are available as to the nature of processes taking place in the liver during thermoregulation. The increase in combustion probably involves mainly nitrogen-free substances. On the other hand, Toenissen has found that surgical exclusion of the central nervous system leads to an increase in protein metabolism, which is bound up with the mechanism of thermoregulation. Besides the liver, the other viscera also participate in the phenomenon of thermoregulation, but their rôle has not been brought to light with sufficient clarity.

Investigations have usually taken the form of acute experiments made on anesthetized animals. This obviously disrupted the normal course of the vital processes and influenced thermogenesis. Anesthesia completely changes the response of an animal to external temperature, and there is even a basis for believing that the specific nature of thermoregulation in warm-blooded animal organisms is destroyed under the influence of anesthetics. This supposition is fully substantiated by the modern theory about the leading rôle of the nervous system in thermoregulation; as is known, anesthetics cause profound changes in the functional state of the nervous system. We therefore thought it of interest

30

to study the participation of the different organs in the total thermogenesis without the use of anesthetics. It was necessary, of course, to select a method that permitted the variations in the temperature of the visceral blood to be measured during the experiments, which would resemble physiological conditions as closely as possible. The method of angiostomy evolved by E. S. London fully satisfies these requirements.

METHOD. We set ourselves the task of studying the angiothermotopography in angiostomized dogs under physiological and pathological conditions.

Temperatures were measured by the thermoelectrical method, which is very convenient from the technical viewpoint, thanks to its accuracy, sensitiveness, speed and the possibility of changing the size and shape of the thermocouples in order to reach parts situated deep in the organism. Claude Bernard made use of the thermoelectrical method for studying the temperature of the blood and various tissues of an organism. He was followed by a number of other investigators. Jeilberg and Lample inserted thermocouples in the vessels and tissues of the cortex of the brain, in which case the animals could be utilized only for two days. Ascher and Schotmann attempted to measure the visceral temperature by means of thermocouples during acute experiments. Rein, who used thermoneedles, found that the blood in the renal veins was warmer than that in the arteries. Hamilton in 1936 determined the temperature of the viscera during the first 48 hours after an operation. Quite recently a substantial success was achieved by Marshak (U.S.S.R.), who evolved a method of inserting thermocouples into the viscera.

We made special angiostomic thermocouples of nickeline and copper. The cold joint of the thermocouple was placed in a special thermostat, where a constant temperature was maintained within the limits of not more than 0.001°. Usually we used five thermocouples, which were connected through a switch with a reflecting galvanometer. All the thermocouples were carefully graduated on the day of each experiment. The experiments were made on angiostomized dogs having cannulas in the portal and hepatic veins. We entered the vessel with an angiostomic needle, being guided by the appearance of blood. The needle was fixed into the cannula and a thermocouple inserted into it in such a way that the thermocouple projected beyond the needle and its warm joint was immersed in the blood stream. We also measured the temperature in the abdominal aorta and the rectum. In order to determine the temperature in the aorta, we inserted a thermocouple into the femoral artery and carefully pushed it upwards against the blood stream; as the thermocouple was 17 cm. long its warm joint reached the abdominal aorta. By means of the switch we were able to measure in turn the temperature of the blood in the portal and the hepatic veins, the aorta and the rectum. Reading of the temperature was carried out according to the deviation of a ray of light reflected from the mirror of the galvanometer onto a scale situated at a distance of 2.5 meters. Each measurement lasted three to five seconds. Thanks to our thermocouples it was possible to measure the temperature of the visceral blood within the limits of 0.02° under physiological conditions without the use of vivisection or anesthetics.

The present report deals with experiments made to study: 1, angiotheromo-topography in normal fasting dogs; 2, angiothermotopography during local cooling and heating of an animal, as well as during certain febrile states.

1. *Angiothermotopography in normal fasting dogs.* Of utmost importance in this respect are the classical works of Claude Bernard, which were carried out as far back as the middle of the 19th century. Contrary to the hypothesis of Lavoisier, which was universally accepted in his time, Claude Bernard showed that venous blood is warmer than arterial blood. Furthermore, he investigated in detail the thermotopography in an organism, employing a long tube fitted with a thermometer or a thermoelectrical needle to enter the left and the right heart, the aorta and the inferior vena cava through the carotid artery and the jugular vein. He found that the blood in the inferior vena cava above the renal vein is of the same temperature as the blood in the corresponding section of the aorta, but that venous blood becomes warmer further on, that is, venous blood cools on the periphery and grows warmer in the viscera. The blood in the portal vein is warmer than that of the aorta, which means that the blood absorbs heat while passing through the intestines. In the hepatic vein the blood is warmer than in the portal vein, thus pointing to thermogenesis in the liver. According to Claude Bernard, hepatic blood is the warmest in the body. Its temperature is 0.6 to 1.6° higher than that of the blood in the portal vein leading to the liver. It should be noted, however, that these were acute experiments.

Few papers on angiothermotopography have been published since the time of Claude Bernard. One of these reports the unexpected results of Miller, Schastnaya and Jutkevich. These authors claim to have found the blood in the hepatic vein to be colder than that in the portal vein, the difference reaching under the conditions of their experiments up to $-1.5°$. According to Chimvicz and Fisikas, the liver has a higher temperature than the other organs.

Our experiments have given very clear and constant results. In the great majority of normal fasting dogs the highest temperature was obtained in the blood of the hepatic vein as compared with all other vascular areas or the rectum. The temperature in the hepatic vein was higher than that in the portal vein by 0.05 to 0.1°. The difference was never very great. Only in exceptional cases was the blood in the portal vein warmer than that in the hepatic vein. This negative difference varied from 0.01° to 0.05° or 0.07°. The temperature of the blood in the abdominal aorta was always lower than in any other of the areas studied. The temperature in the rectum was usually close to that in the portal vein.

The difference in temperature between the portal and hepatic veins characterizes the thermogenesis in the liver; the difference in temperature between the aorta and the portal vein reflects the thermogenesis in the intestines. Our experiments have shown clear cut evidence of intestinal thermogenesis. This increase usually varied from 0.1 to 0.3° (see table 1). Claude Bernard noted this fact, but did not dwell on it, and recent literature does not mention it. Incidentally, the intestinal tract, with its great length and exceedingly active metabolism, cannot but play an important part in thermogenesis in the body.

Thus, by comparing our results with those of Claude Bernard, we see that the values for intestinal thermogenesis are almost coincident. As for hepatic thermogenesis, our experiments have given considerably lower figures than those obtained by Claude Bernard.

The scale of our experiments, the accuracy of our methods and the way in which the physiological conditions were reproduced warrant the belief that our data are closer to reality.

2. *Experimental cooling and heating of animals.* Having studied thermotopography in normal dogs, we made some experiments on artificially cooled and heated animals. The experiments were carried out on dogs in winter conditions 14 to 16 hours after their last feeding. The temperature was measured at the same points described above. After stable temperature values had been obtained,

TABLE 1

Variations in the temperature of the blood and the rectum of normal fasting dogs

AORTA	PORTAL VEIN	HEPATIC VEIN	RECTUM	LIVER	INTESTINES
39.55	39.78	39.93	39.83	+0.15	+0.23
39.02	39.20	39.24	39.09	+0.04	+0.18
39.36	39.54	39.56	39.43	+0.02	+0.18
39.00	39.07	39.20	38.81	+0.13	+0.07
39.14	39.18	39.26	38.99	+0.08	+0.04
37.90	38.25		38.14		+0.35
	39.50	39.55	39.56	+0.05	
38.75	39.02		39.00		+0.27
39.22	39.32		39.38		+0.10
	39.92	39.96	39.90	+0.04	
39.79	39.95		39.74		+0.16
38.56	38.82		38.95		+0.26
	39.87	39.94	39.64	+0.07	
	38.83	38.77	38.80	−0.06	
	39.91	38.84		−0.07	
	38.87	38.95	38.82	+0.08	
39.63	39.91		39.85		+0.28

the animals were cooled by applying ice or towels soaked in cold water onto a large area of the skin on the back and hind legs. The cooling usually lasted from 30 to 90 minutes and gave a very clear-cut effect. The temperature in the rectum always fell to a certain extent during the first 5 to 10 or 15 minutes. During the same time the temperature of other parts of the animal underwent significant changes. A distinguishing feature of these changes was a comparative rise in the temperature of the blood in the hepatic vein in relation to the portal vein and the aorta. Thus the difference in temperatures between the hepatic veins and the portal vein and, especially, the aorta increased considerably. If prior to cooling there was a difference of 0.06 to 0.1°, it increased 3 to 6 times after cooling. When the cooling agent was withdrawn, hepatic thermogenesis quickly returned to the original level. Restoration and reduction of the difference in

temperature between the blood flow into and out of the liver proceeded more rapidly if the animal was warmed up under a special electric heater.

In analyzing our data from twenty-five experiments on ten dogs, it is necessary to establish which processes are reflected in the variations in the difference in temperature between the inflowing and the outflowing blood. It goes without saying that this difference depends on two factors, i.e., thermogenesis in the liver and its blood supply. It must be assumed that the experimental cooling led to a dislocation in the blood supply to different parts of the organism so that circulation was delayed on the periphery and accelerated in the viscera. If we assume that thermogenesis in the liver is not increased under local cooling, then acceleration of the blood circulation should reduce the difference in temperatures between the blood flowing into and out of the liver. Yet we never recorded such a reduction. On the contrary, the temperature of the hepatic blood was always much higher as compared with that of the blood in the portal vein and the aorta. In
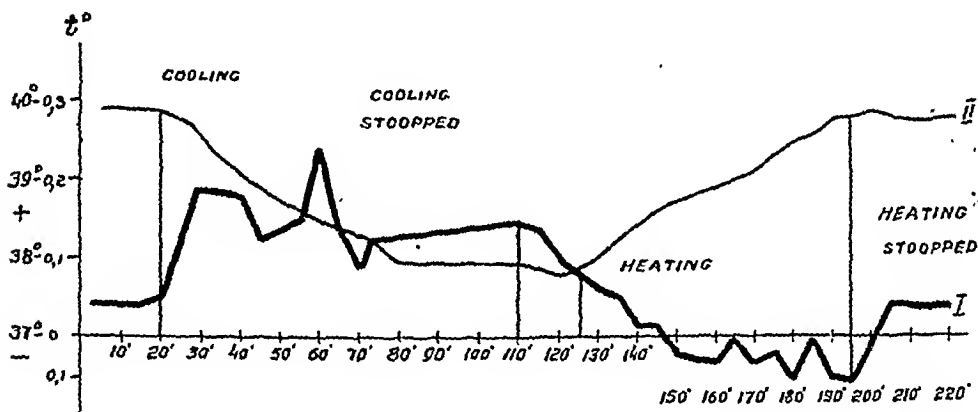
Fig. 1. Thermal reactions of the liver to cooling and heating the animal body. Curve I represents the temperature of the liver; curve II that of the rectum.

our opinion, these results indicate that the liver reacts to cooling by increased thermogenesis. Such a reaction is a reflection of chemical thermoregulation by the liver. In view of the speed with which it begins, it may be thought that the increase in thermogenesis is of a reflex nature.

The experiments described above demonstrate the adaptation of the metabolic processes of the liver to a change in external temperature.

We next proceeded to study angiothermotopography under conditions of intrinsic overheating, i.e., during a febrile process. This investigation comprised a number of experiments with the transfusion of homogeneous and heterogeneous blood and the induction of anaphylactic shock.

3. *Experimental fever. a. Homogeneous transfusion.* In this series of experiments the angiostomized dogs were injected with homogeneous blood of the same group to the amount of 10 to 15 cc. per kilogram of body weight. Altogether 12 experiments were made. In most cases the general temperature of the dog rose by 0.3 to 1.4° during the experiment, which lasted 2 to 4 hours; in other words,

there was a slight fever.   The rise in the temperature in the rectum and of the blood was invariably preceded by a drop in the temperature in all other areas studied.   As we suspected that this initial drop in temperature, which lasted but a few minutes after the transfusion, was due to the use of colder blood, in all subsequent experiments the blood was specially heated to body temperature.   This, however, did not alter our results.   We shall subsequently dwell in detail on this phenomenon, which occurred very regularly.   It is interesting to note that in spite of the general rise in temperature, angiothermotopography in the animal was not changed; the relation between the temperatures in the various vessels remained the same as before the experiment.

Analysis of the data secured in these experiments shows no abnormality in angiothermotopography.   Intestinal thermogenesis also remains practically unchanged.   In view of this, we deem it possible that the febrile changes after transfusion are explained by a change in physical rather than chemical thermoregulation.

b. *Heterogeneous transfusion.*   In this series of experiments the dogs were injected with the blood of another species.   We used goat's blood to the amount of 5 cc. per kilogram of body weight.   In almost all of the 15 experiments made in this manner a considerable rise in rectal temperature was observed, amounting usually to 0.3 to 1.8° in the first two or three hours after the transfusion.   Just as in the preceding series the temperature fell for a few minutes prior to the development of the febrile state.   An especially significant drop in temperature was invariably noted in the aorta.   Measurement of the blood pressure during the experiments with heterogeneous transfusion showed that it decreased simultaneously with the temperature in all the areas studied.   This throws light on the origin of the initial drop in temperature.   It must be assumed that the shock induced by heterogeneous transfusion is inevitably accompanied by retardation of the flow of blood and consequently by a cooling of considerable amounts of blood in the surface parts of the organism.   Thus the right heart is supplied with cooler blood, which continues to lose heat while it passes through the lesser circle. Herein lies the explanation for the drop in temperature observed during the first minutes after transfusion and especially noticeable in the arterial system.   This signifies that the changes in temperature developing during the period of the shock induced by heterogeneous transfusion are due solely to the changes in the circulation of the blood.   In those cases when heterogeneous transfusion did not cause a decrease in blood pressure, followed by retardation of the circulation, we never observed any drop in temperature.   On the contrary, the temperature began to rise during the very first minutes, particularly that of the blood in the aorta (see fig. 2).

If we draw a parallel between these observations and the drop in the temperature brought about by homogeneous transfusion, we may conclude that in the latter case there also develop hemodynamic deviations which cannot be detected by the relatively crude and inert measurements of the blood pressure but might be revealed by a more delicate method, i.e., by determining the velocity of the blood flow.   When analyzing our data on heterogenous transfusion, we may

speak about two stages in the dynamics of the temperature shifts: 1, a stage related exclusively to the changes in circulation, and 2, the subsequent stage of restoration of blood pressure and velocity of circulation.

During the first stage, when blood pressure falls, the temperature drops in all the areas studied and also the difference in temperature between the aorta and the portal vein increases. But have we a right to assume that this increase is due to heightened thermogenesis in the intestines? In this stage circulation is retarded and the blood stagnates in the viscera, which must inevitably lead to a corresponding increase in the arterio-venous difference. Thus the increase in the difference in temperature between the aorta and the portal vein during the first stage depends entirely on circulatory factors.
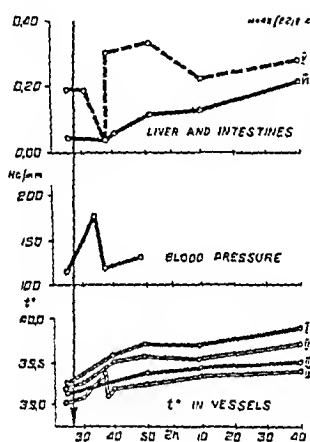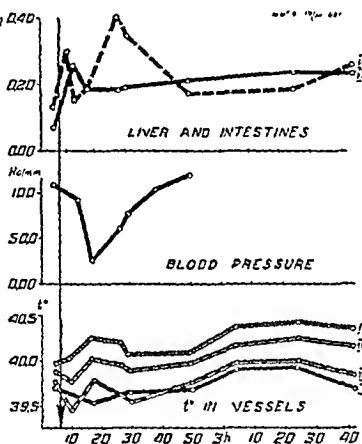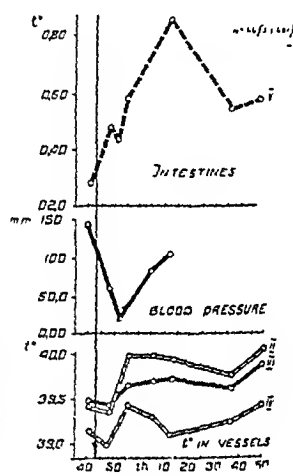
Fig. 2   Fig. 3   Fig. 4

Fig. 2. Thermal reactions to heterogeneous transfusion. I, temperature of blood in hepatic vein; II, in portal vein; III, rectal temperature; IV, in femoral artery; V, in intestinal vessels (i.e. difference between temperature in portal vein and femoral artery); VI, in liver (i.e. difference between temperature in portal and hepatic veins).

Fig. 3. Thermal reactions in heterogeneous transfusion. I, temperature of blood in hepatic vein; II, in portal vein; III, rectal temperature; IV, in femoral artery; V, in liver.

Fig. 4. Thermal reactions in anaphylactic shock. II, temperature of blood in portal vein; III, in femoral artery; IV, rectal temperature.

Of considerable interest to us is the second stage, when the hemodynamics are restored both with respect to blood pressure and the velocity and time of circulation. Turning to the changes in temperature in the various vascular areas, we see that the difference in temperature between the portal vein and the arteries as well as between the hepatic vein, on the one hand, and the aorta and the portal vein, on the other, continues to increase instead of diminishing. As circulatory influence is excluded in this stage this must be an expression of a genuine increase in visceral thermogenesis. Thermogenesis in the liver as well as the intestines rises several times as compared with normal conditions.

The foregoing experiments clearly demonstrate that the increase in visceral thermogenesis coincides in time with the rise in rectal temperature. Hence the

conclusion may be made that thermogenesis in viscera (liver and intestines) plays a part in the febrile process which inevitably accompanies the introduction of a foreign protein. The mechanism of this action is obscure; of some importance are probably the products of the disintegration of the proteins present in the transfused blood. Such a disintegration is the result of the colloidoclastic crises commonly accompanying blood transfusion. The newly formed substances activate visceral metabolism and thus increase thermogenesis. After homogeneous transfusion the products of protein disintegration accumulate probably in much smaller amounts, insufficient to activate visceral thermogenesis.

Anaphylactic shock. The anaphylactic shock was induced in several angiostomized dogs temperature measurements as above described. The results were similar to these obtained after heterogeneous transfusion, except that the variations in temperature were more clear cut. As in the case of homogeneous and heterogeneous transfusion, the temperature dropped in all vascular areas, especially in the aorta during the first few minutes following the second injection of foreign blood (see fig. 3). This drop in temperature coincided with the fall in the blood pressure. During this period the increase in the difference in temperature between the aorta and the portal vein was due to changes in circulation. But after the circulation had been restored the difference in temperature continued to rise, owing to a genuine increase in thermogenesis of the intestines. Intestinal thermogenesis reached very high values never obtained under other experimental conditions. Besides the increase in intestinal thermogenesis, a rise in rectal temperature was observed, pointing to the conclusion that heightened thermogenesis in the viscera is one of the factors determining the febrile state after anaphylactic shock.

SUMMARY

1. Thermogenesis in the viscera (liver and intestines) was studied in normal, artificially cooled and heated animals, as well as during febrile states caused by homogeneous and heterogeneous blood transfusions.

2. The experiments were made on angiostomized dogs with cannulas in the portal and hepatic veins; in each animal the rectal temperature and the temperature of the blood in the abdominal aorta and the portal and hepatic veins were measured.

3. The thermoelectric method used throughout the experiments detected changes in temperature with sufficient accuracy and made it possible to study thermotopography in the organism.

4. In normal fasting dogs the lowest temperature of the blood was found in the aorta and the highest in the hepatic vein.

5. The experiments have shown important thermogenesis in the intestines.

6. When the animals were cooled by the application of ice to the skin, the difference in temperature between the blood in the hepatic and the portal veins increased, i.e., there was a rise in hepatic heat production (three to six times the original level), providing the blood flow was accelerated; when the animal was overheated, the reverse took place.

7. Homogeneous blood transfusion did not bring about noticeable changes in thermogenesis in the liver and the intestines, despite a systemic rise in temperature.

8. The febrile state caused by heterogeneous transfusion is accompanied by a noticeable increase in thermogenesis in the liver and intestines. A comparative study of the hemodynamic variations has shown that the increase in hepatic and intestinal thermogenesis cannot be explained by the influence of circulatory factors.

9. After anaphylactic shock, the same changes were observed as in the case of heterogeneous transfusion, but the variations in temperature were much greater, especially with respect to the intestines.

10. The increase in visceral thermogenesis proceeds parallel to the systemic temperature reaction, i.e., the liver and the intestines participate in determining the febrile process accompanying the transfusion of foreign blood.

11. The use of the method of E. I. London for studying visceral thermogenesis in angiostomized dogs considerably widens the sphere of its application and promises to give results of great practical and theoretical interest.

REFERENCES

(1) Isenschmid, R.   Handbuch d. norm. u. pathol. Physiologie, 17: Correlationen III, 1926.
(2) Bernard, C.   Leçons sur la chaleur animale.   Paris, 1877.
(3) Freund, N.   Handbuch d. norm. u. pathol. Physiol. 17: Correlationen III, 1926.
(4) Cavasin.   Zentralbl. f. Physiol. 8: 1894.
(5) Hirsch and O. Mueller.   Arch. f. klin. Med. 75: 1903.
(6) Hirsch and Rolly.   Arch. f. klin. Med. 75: 1903.
(7) Orbeli, L. A. and A. V. Jonkikh.   Fiziol. Zhurn. USSR, 24: 249, 1938.
(8) Krehl, L.   Handb. d. allg. Pathologie v. Krehl-Marschaud, 4: 1924.
(9) Dubois, R.   Ann. de l'univ. de Lyon, Paris, 1895.
(10) Gessler.   Ergebn. d. Physiol. 26: 1928.
(11) Richet, C.   Dictionnaire de Physiologie.   Bd. 3, Ar. Chaleur, Paris, 1898.
(12) Marshak.   Arkhiv. biolog. nauk, 55: no. 3, 1939.

THE RELATION BETWEEN THE PHYSICAL PROPERTIES OF ELECTRIC CURRENTS AND THEIR ELECTRONARCOTIC ACTION[1]

A. van HARREVELD, M. S. PLESSET and C. A. G. WIERSMA

*From the William G. Kerckhoff Laboratories of the Biological Sciences,
California Institute of Technology, Pasadena*

The fact that unidirectional pulse currents applied to the central nervous system of mammals (rabbits and dogs) can cause readily reversible narcotic states was described by Leduc in a series of papers, the first of which, containing the essentials, appeared in 1902. This observation was confirmed by many authors (see for instance von Neergard, 1922; Zimmermann, 1929). In most of these experiments, one electrode (cathode) was placed on the head, the other (anode) on the sacrum. Leduc maintained that electronarcosis (en.) could be produced with pulse currents only; however, a similar state was obtained by applying direct current (Tschagowetz, 1912; Silver, 1939) and alternating current (van Harreveld and Kok, 1934a).

The modern therapeutic use of electric current applied to the brain, known as electroshock, has renewed the interest in such procedures as en. Electroshock therapy consists in the application of a strong current for 0.1 to 1.0 sec.; for en. a much weaker current is used, which flows during the entire experiment.

In the present investigation, the effects of pulse currents of various pulse durations and frequencies, alternating currents of various frequencies, and direct current have been compared. An attempt has been made to determine which of the several possible effects of electrical current on the central nervous system is the cause of en.

METHOD. For the production of pulse currents a thyratron stimulator has been used, capable of producing unidirectional square pulses of a wide variety of frequencies and durations, both of which could be changed independently. This current was used to drive a 50 watt amplifier.[2] Sinusoidal alternating current was obtained from an audio frequency generator, the output of which was amplified by the same 50 watt amplifier. A calibrated oscilloscope was used to measure voltage and current for pulse as well as for alternating current. Furthermore the current shape could thus be checked at any time. The direct current, obtained from the laboratory supply, had a ripple which was too small (about 1.5 per cent) to be of physiological importance.

The electrodes used in the pulse and alternating current experiments consisted of copper discs about 2 cm. in diameter, covered with gauze soaked in concentrated sodium chloride solution. The contact between electrodes and skin, from

which the hair was clipped, was secured with an electrode paste. For direct current experiments non-polarizable electrodes were used. Each electrode consisted of a shallow lucite box (2.5 cm. in diameter) containing a copper electrode; the box was filled with saturated copper sulfate solution and was covered with an animal membrane (bladder or intestine). Electrode paste was again used between electrode and skin.

In all the present experiments the electrodes have been placed on the temples directly posterior to the eyes. This placement has the advantage that the current remains confined to the head, and thus produces less disturbing effects on peripheral structures. The electrodes were fastened on the head with bandages.

Dogs have been used in all the experiments here reported.

Symptoms of electronarcosis. The most satisfactory way of producing en. is to apply a relatively strong current (e.g., 60 cycle alternating current of about 300 ma) for about 30 sec., which then is decreased to a lower level. Immediately upon applying the strong current, the animal falls down, its legs in a flexed position. During the next few seconds, the extremities are almost toneless, but after 5 to 10 sec. a strong extensor spasm develops in the legs which are stretched backwards. There is complete respiratory arrest. This state then remains unchanged during the application of the strong current. Often urination occurs and sometimes there are bowel movements.

After 30 sec. the current is decreased to a value, the "narcosis level", at which sufficient respiration becomes possible; this level depends on the type of current used (for 60 cycle a.c. it is 30–60 ma), on the individual reaction of the animal, and probably on the placement of the electrodes. The decrease of the current causes relaxation of the extensor spasm and appearance of clonic twitches. The first respiration will occur 50 to 70 sec. after the beginning of the experiment. If the current is too strong, the return of respiration is delayed and the current has to be diminished. After regular respiration has set in, the animal lies quietly and without tone in its muscles although light clonic twitches may proceed for some minutes. The symptoms can subsequently develop in two directions, which will be designated as the narcotic and the kinetic type of en.

In the narcotic type, the animal remains quiet, with a slow and deep respiration, which is often labored because of glottis contraction (von Neergard, 1922). The heart rate is low, about half normal, due to vagus stimulation (van Harreveld and Kok, 1934a). Except for respiratory movements and sometimes occasional clonic twitches, the animal shows no spontaneous activity. Positive and negative supporting reactions are present when the legs are placed in the proper positions. However, when the animal is placed on its feet, the legs do not gain sufficient supporting tone and the animal falls down as in deep narcosis. The tail is without pronounced tone. In some dogs, tone in the abductor muscles of the thigh is observed. The head is extended dorsally and the eyes are closed tightly; the latter symptoms are probably caused by a direct stimulation of the muscles involved. Tendon reflexes like the knee jerk are not disturbed. No righting reflexes can be elicited. Pinching or pricking the skin does not cause

any reaction. Pressure on the eye ball through the closed eye lids is often without effect, but sometimes causes defecation or irregularities in the respiration. From time to time there is a bowel movement. The narcotic type of en. can be maintained for many hours.

Increase of the current beyond the value necessary to maintain en. causes extensor tone in the extremities independent of the position of the legs. Respiration is more difficult and becomes insufficient.

As the current is decreased below the narcosis level, the supporting reaction becomes stronger and stronger until finally the animal can stand. Even in this state, there is no return of spontaneous movements, of righting reflexes, or of reactions to skin stimuli. When the legs are placed in abnormal positions, no corrective movements are made. These cataleptic symptoms have been described before (Keller, 1931; van Harreveld and Kok, 1934b).

When the current is cut off during the narcotic type of en. the animal recovers in a few minutes, usually passing through a cataleptic state.

The kinetic type of en. is of a much less quiet nature, and is characterized by frequent righting attempts which are usually unsuccessful and which can develop into violent and disordered hyperkinesis of head and extremities, often accompanied with yelling and whining. Between righting attempts the animal is quiet, but righting and hyperkinesis can be instigated by stimuli. The most effective is pressure on the eye ball; also effective is moving the animal, thus stimulating the vestibular apparatus and perhaps other sensory systems. Sometimes skin stimuli are sufficient. The heart and respiration rate are higher than in the narcotic type, usually even higher than normal.

Cutting off the current during the kinetic type of en. is followed by immediate recovery without cataleptic symptoms. After awakening, the animal is often somewhat excited for a short time.

Although the purely kinetic type is rare, some kinetic symptoms are often found during the narcotic type; e.g., an occasional weak righting attempt, yelling, or movements on eye pressure.

The type of electronarcosis which will be produced is, for the most part, an individual reaction. However, after a long series of experiments on the same animal, kinetic symptoms may appear or become aggravated. Also, kinetic symptoms may become more and more apparent during a single prolonged en.

Some authors (Sack and Koch, 1933; Koch and Sack, 1933) have not been able to repeat Leduc's experiments; they observed only unquiet states. It is possible that these authors by their choice of animals or by their technique of current application obtained predominantly the kinetic type of en.

In the following quantitative investigations, only dogs with a tendency to the narcotic type of en. have been used.

Electronarcosis with pulse currents of various durations. Pulse currents of frequency 120/sec. were used throughout these experiments. For the first 30 sec., pulses of 0.3 millisec. duration and 600 to 750 ma strength were used. Electronarcosis level for pulse currents of this duration was 80 to 125 ma. After 10 to 15 minutes when the animal had reached a steady state, the duration of the

pulses was changed, keeping the frequency constant. After each change, the current strength was so adjusted as to produce the same depth of en. The depth of en. chosen was such that when the animal was placed on its legs only a trace of supporting reaction was present. The necessary current strength for 6 to 8 values of pulse durations could thus be determined in the course of an hour. On varying the pulse durations between 2 and 0.5 millisec., hardly any current adjustments had to be made. With pulse durations shorter than 0.5 millisec., the current had to be increased to maintain the same depth of en. This increase is exponential with the decrease in pulse duration. A strength-duration curve for en. can thus be plotted (fig. 1). From such curves, rheobase and chronaxie can be determined, and these values obtained from 6 experiments on 6 different ani-
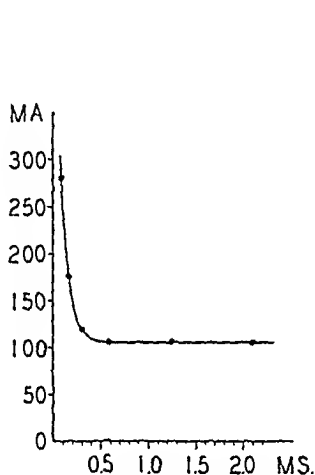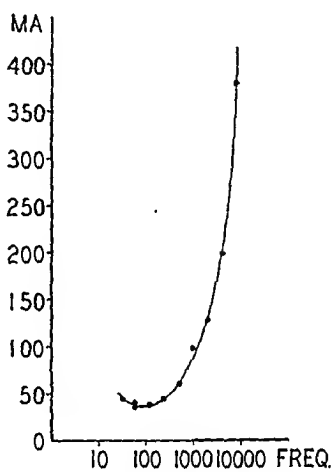
Fig. 1   Fig. 2

Fig. 1. Strength-duration curve of electronarcosis produced with unidirectional square pulses. The top current intensity of the pulses measured in milliamperes is plotted as ordinate against the duration of the pulses in milliseconds as abscissa. All points of the curve refer to the same narcosis depth. The pulse frequency in the experiment was 120/sec.

Fig. 2. Strength-frequency curve of electronarcosis produced with sinusoidal alternating currents. The root mean square intensity of the current measured in milliamperes is plotted against the logarithm of the frequency. All points of the curve refer to the same narcosis depth.

mals have been collected in table 1. No difference in symptoms was observed with the pulse durations used (between 0.08 and 2 millisec.).

Electronarcosis with pulse currents of various frequencies. Two procedures have been used to examine the effect of varying the pulse frequency. In part of the experiments, en. was initiated with pulse current of frequency 120/sec. and of short duration (0.17 millisec.). After the animal had reached a steady state, the frequency was changed, and the current was adjusted so as to produce the same reference depth as described in the pulse duration experiments. Any sudden increase of pulse frequency causes a short period of excitement. Up to a frequency of 300–400/sec., no changes occur in the symptoms of en. With frequencies above 500/sec., en. becomes less quiet in most animals. Twitches and

contortions appear, and symptoms of the kinetic type have a tendency to break through. These unquiet symptoms are especially pronounced in the frequency range between 500 and 1000/sec. In a few animals, however, any frequency produced quiet en. Even at the highest frequency practicable with a pulse duration of 0.17 millisec. (about 3000/sec.), en. has been maintained.

Very little current adjustment had to be made to maintain the same depth of en. to approximately 1000 pulses/sec. At higher frequencies, however, a moderate current increase was necessary (at 3000/sec. the current was about doubled). The cause of this rise in current intensity is probably due to polarization effects.

In similar experiments, the pulse frequency has been decreased after establishing en. at frequency 120/sec. It has been possible to go down as far as 15 to 20 pulses per sec. and once even to 5 pulses per sec. without awakening the animal. At the lowest frequencies, tremblings and twitches occur which are synchronous with the pulses. There is also a tendency to develop kinetic symptoms, especially righting. No significant current adjustment was necessary in these experiments.

<div align="center">TABLE 1</div>

<div align="center">*Rheobase and chronaxie of electronarcosis*</div>

Number of animal	1	2	3	4	5	6
Rheobase in ma	105	85	105	100	75	140
Chronaxie (millisec.)	0.14	0.16	0.14	0.14	0.16	0.12

The other way in which the influence of frequency has been examined was to produce a series of short en. (of about 15 min. duration) with pulse currents of various frequencies. This type of experiment has been performed on two animals using the frequencies 30, 60, 120, 240, 480, 1080 and 2160/sec. Again, en. with pulse frequency 480/sec. and above were less quiet than with the lower frequencies.

It thus seems possible to maintain as well as to produce en. with pulse currents of a wide range of frequencies.

Electronarcosis with alternating currents of various frequencies. A set of experiments, similar to those described for pulse current, has been performed with sinusoidal alternating current of various frequencies. In part of the experiments, en. was initiated in the usual way with 60 cycle current. The frequency was then changed and the current strength was adjusted so as to produce the reference depth described above. The symptoms of en. produced with alternating current are identical with those produced with pulse current.

A range of frequencies between 30 and 8000/sec. has been examined. Electronarcosis was less quiet at frequencies above 500, especially in the range between 500 and 2000/sec. The current necessary to maintain the reference depth at these various frequencies had a minimum at about 100 cycles/sec. A slight current increase was necessary when the frequency was decreased to 30/sec. As the

frequency was increased, however, the current had to be increased considerably. At 8000 cycles/sec., for instance, about 10 times as much current had to be applied as at 100 cycles/sec.   From these data, a strength-frequency curve can be plotted (fig. 2).   This curve resembles strikingly the pararesonance curves determined in a similar way by stimulating peripheral nerves with alternating currents of various frequencies (Coppée, 1934).

In one animal a series of 15 minute electronarcoses was given, applying alternating currents of various frequencies from the start.   In this way, the frequencies 30, 60, 120, 240, 500, 1000, 2000 and 4000 cycles/sec. have been examined.   Electronarcosis could be produced with all these frequencies.   The strength-frequency curve, plotted from this series of experiments, corresponds well with the strength-frequency curve of the same animal which was determined during a single session.

It can be concluded that en. can be maintained and produced with alternating current of a wide range of frequencies.

Experiments with direct current.   In the first attempts to produce en. with direct current, a strong current (300–500 ma) was applied for 30 sec. which was then diminished to a lower level (150–200 ma).   It was found, however, that even a current of 500 ma maintained during the entire experiment causes the same symptoms and does not endanger life.   It is not feasible to use higher currents, since a current of 500 ma has a considerable heating power (approximately 50 watts).

Immediately after making the current the animal falls down in a flexed position.   Usually this is followed after a few seconds by stretching of the legs.   Ten to 15 seconds later, clonic twitches appear which go on for 15 to 30 sec.   A contortion of the neck toward the positive pole develops.   Respiration returns after 30–50 sec. irrespective of current strength.   Except for the contortions, these initial symptoms resemble a milder and abbreviated form of those following the application of pulse or alternating current.

The contortion of the head towards the anode remains for the duration of current application.   It is probably caused by direct current stimulation of the labyrinths (galvanic labyrinth reaction).   The head contortion brings into play a series of other reflexes which result in rolling movements (Magnus, 1924).   These rolling movements make it difficult to observe other symptoms.   However, skin stimuli sometimes cause the flexion reflex, and pressure on the eye ball increases the rolling movements.

After switching off the current, the animal rolls for some minutes toward the negative pole, and shows a head contortion in that direction.   The recovery usually proceeds more slowly than after the application of pulse or alternating current.

The application of direct current to the head causes symptoms which are partly specific direct current effects (galvanic labyrinth reaction, rolling movements).   There is probably in addition an electronarcotic effect which, though much higher currents were used, was much weaker than that produced by pulse or alternating current.

DISCUSSION. The strength-duration curves of electronarcosis, which have been obtained by applying repeated pulses, bear a striking resemblance to those obtained by applying single pulses to peripheral nerves. It is therefore strongly indicated that en. is due to the stimulating properties of the current. The short chronaxie of electronarcosis (0.12–0.16 millisec.) indicates that highly excitable structures, nerve fibers or cells, are stimulated. This view is supported by the resemblance of the strength-frequency curve of en. and the pararesonance curves determined in peripheral nerves. The rise in intensity for high frequencies, necessitated by decreased duration of the stimulating current peaks, is clearly demonstrated by the data; the rise in intensity for low frequencies which is a consequence of accommodation is only indicated, probably because the frequency range could not be extended sufficiently low. Thus the structures affected by alternating current in en. show the same features of excitation and accommodation as do peripheral nerves (Hill, 1936).

Although direct current of sufficient intensity can produce repeated discharges in nervous tissue, the stimulating power is much smaller than that of pulse or alternating current because of accommodation. Thus the weak electronarcotic effect of direct current also supports the view that en. is due to the stimulating properties of the current.

Two mechanisms can be considered for the explanation of the depression of central nervous activity by stimulation. The diffuse application of current to large parts of the central nervous system will stimulate structures with excitatory as well as those with inhibitory function. It is possible that, when these are stimulated simultaneously, inhibition prevails. The inhibition of the knee jerk by a homolateral pain stimulus is an instance of the fact that simultaneous stimulation of excitatory and inhibitory pathways results in absence of activity. Thus, according to this view, the normal central inhibition, irrespective of its mechanism, is responsible for en.

The other possibility is that en. is due to Wedensky inhibition, and thus is due to the fact that impulses are set up at a frequency too high for the structures involved. The comparable effects of pulse and alternating currents of widely varying frequencies can be considered as an argument against this view since Wedensky inhibition would be produced with greater ease by high than by low frequency currents. Since reflex inhibition is sometimes explained on the basis of Wedensky inhibition, the difference between the two explanations of en. may seem very small indeed.

SUMMARY

1. The effects of electrical currents applied to the brains of dogs with electrodes placed on the temples have been studied. In addition to a type of electronarcosis (en.) which resembles chemical narcosis and was described by Leduc, another unquiet type, characterized by righting reflexes and hyperkinesis has been observed. The quiet type has been designated as the narcotic, the unquiet type as the kinetic type of en. Pulse as well as alternating current can produce both types of en. and which type appears is for the most part an individual reaction.

2. The relation between the pulse duration and the pulse strength necessary to produce the same depth of en. has been determined; this relation resembles closely the strength-duration curves of peripheral nerves.

3. Electronarcosis has been produced with a wide variety of frequencies of pulse and alternating current. For alternating current, the relation between the frequency and the current strength necessary to maintain the same depth of en. has been determined; this relation resembles the pararesonance curves obtained for peripheral nerves.

4. The electronarcotic effect of direct current is small as compared with that of pulse or alternating current.

5. It is concluded from 2, 3 and 4 that en. is due to the stimulating properties of the current applied.

REFERENCES

COPPÉE , G. Arch. int. Physiol. **40**: 1, 1934.
HARREVELD, A. VAN AND D. J. KOK. Arch. néerl. Physiol. **19**: 24, 1934a.
        Arch. néerl. Physiol. **19**: 265, 1934b.
HILL, A. V. Proc. Roy. Soc., London, ser. B. **119**: 305, 1936.
KELLER, C. J. Acta brevia Neerl. Physiol. **1**: 100, 1931.
KOCH, H. AND G. SACK. Ztschr. ges. exper. Med. **90**: 365, 1933.
LEDUC, S. C. R. Acad. Sci., Paris **135**: 199, 1902.
MAGNUS, R. Körperstellung. Springer, Berlin, 1924.
NEERGARD, K. VON. Arch. klin. Chir. **122**: 100, 1922.
SACK, G. AND H. KOCH. Ztschr. ges. exper. Med. **90**: 349, 1933.
SILVER, M. L. Proc. Soc. exper. Biol. **41**: 650, 1939.
TSCHAGOWETZ, W. Pflüger's Arch. **146**: 567, 1912.
ZIMMERMANN, J. Münch. tierärztl. Wchnschr. **80**: 121, 141, 155, 1929.

THE SECRETION OF RENIN BY THE INTACT KIDNEY

F. HUIDOBRO AND E. BRAUN-MENENDEZ

From the Institute of Physiology, Faculty of Medical Sciences, University of Buenos Aires

Hypertension which follows constriction of the renal arteries is accompanied by the liberation of renin into the blood by the ischemic kidney (9, 4, 14, 3). Renin, which appears to be an enzyme, acts on a blood globulin (hypertensin precursor) and gives rise to hypertensin, a substance which by its vasoconstrictor action and its action on the heart, produces a rise in blood pressure (12, 1, 2, 13).

Since the discovery was made that the ischemic kidney liberates a pressor substance into the blood the question arose whether this pressor substance resulted from the pathologic condition of the kidney or whether it was produced already in small amounts by the normal kidney. Some observations afford indirect evidence in favor of a normal secretion of renin. Dogs nephrectomized 24 or 48 hours previously are more sensitive to the pressor action of renin (17, 11, 18, 5, 15, 8, 16). In these animals an increase in the concentration of hypertensin precursor has been found (13) as well as a hypersensitivity of the vessels to hypertensin (15, 8, 16). Both these findings would indicate that the kidney secretes normally minimal amounts of renin.

The graft of a normal kidney into the neck of a normal or recently nephrectomized dog does not produce a rise in blood pressure (9, 4, 6). A very definite one is observed, however, if the receptor dog has been nephrectomized 48 hours previously (6). The hypersensitivity of these animals reveals the presence of renin in the venous blood of even a normal kidney. The obvious objection is that the total ischemia to which the kidney has been subjected from the moment at which it is excised to the moment at which the graft is completed may cause the accumulation of renin in the kidney. If the kidney is grafted into a normal dog no rise in pressure is elicited because the amount of renin liberated by the kidney is extremely small. In a dog nephrectomized 48 hours previously, however, where the sensitivity to the pressor action of renin is greater, a definite rise is observed.

Though these findings suggest that the intact normal kidney perhaps secretes renin into the blood, its first clear demonstration was made by Houssay et al. (7). Renin was estimated in systemic blood of dogs after its intravenous injection. The concentration of renin usually reached a peak and then gradually diminished until it disappeared in from 1 to 4 hours. In two of the control dogs in which the kidneys had been manipulated but not removed, the concentration of renin in the blood began to increase. These dogs presented the picture of shock with low blood pressure and sighing respiration. In view of this observation which suggested that in shock the kidney might liberate renin, a systematic study was thought advisable.

The experiments which we will report show that hypotension even of short

47

duration causes the intact normal kidney to secrete renin into the blood in amounts sufficient to be estimated in the general blood of normal anesthetized dogs. The inference is drawn that the kidney participates in the regulation of arterial blood pressure and that renin is one of the many physiologic substances which the body uses to maintain its constant state.

MATERIAL AND METHODS. Dogs anesthetized with nembutal (0.04 gram per kilo) or amytal (0.05 gram per kilo) were used. Renin was estimated in the blood by the direct method of Leloir et al. (10). This method is based on the estimation of the pressor action of hypertensin formed when blood plasma which contains renin and which has been freed of hypertensinase by acidification is incubated for 2 hours at 37°C. with hypertensinase-free bovine plasma.

EXPERIMENTS AND RESULTS. A. *Hemorrhage: Group I.* Normal dogs weighing from 8 to 33 kilos and anesthetized by the intraperitoneal administration of nembutal were used. The blood pressure was determined by a cannula introduced into the carotid artery and connected to a mercury manometer. A sample of arterial blood was obtained for the determination of renin. Blood was then withdrawn from the femoral artery each ten minutes in an amount corresponding to 1 per cent of the body weight until 4 per cent was reached. At intervals varying from 30 to 120 minutes after this moment, new samples of blood were obtained for the determination of renin. The results are shown in table 1. In all, 16 experiments were made. Six of these were negative, that is to say, no renin was detected in the samples of blood obtained after hemorrhage. As can easily be seen, the causes of this failure were: *a*, the amount of plasma used for the determination was too small (6 to 8 cc.) in dogs 2, 3, 4, 8, 9, and 10; *b*, the time elapsed since the hemorrhage was complete was in most cases too short (30 min. for dogs 2, 3, and 4 and 40 for dog 9). Furthermore, in dog 8 the blood pressure remained at a level of 90 mm. Hg. The other 10 dogs all had renin in the systemic blood following varying periods of hypotension due to hemorrhage. In some cases (dogs 11 and 14) it can be seen that the concentration of renin in the blood gradually rose. To be sure that the pressor action obtained by the injection of the extract of plasma incubated with hypertensin precursor for 2 hours at 37°C. was really due to renin, all the controls recommended by Leloir et al. (10) and others were prepared.

Group II. That the kidney was responsible for the appearance of renin in the systemic blood of the dogs after hemorrhage was shown by the negative results obtained in 6 nephrectomized dogs subjected to the same procedure (table 1).

Group III. In 3 dogs the kidneys were completely denervated before hemorrhage. This was accomplished by externalizing the kidney through the lumbar route, cutting all visible nerves, and rubbing vein and artery with a gauze soaked in alcohol. Hypotension was then produced by removing blood in the same manner as already described. Renin was found in the blood of these dogs in amounts comparable to those found in the normal intact dogs of group I (fig. 1).

Group IV. To see whether arterial hypotension produced by some other method would produce the liberation of renin by the kidney, 2 dogs with the adrenals

removed and anesthetized with nembutal were subjected to repeated manipulation of the small intestine. The blood pressure fell progressively (fig. 2) while the renin content of the blood rose. Two normal control dogs failed to show either hypotension or renin in the systemic blood as a result of the anesthesia.

Group V. Experiments were made with the aim of destroying as much as possible the tubular cells to see whether in these conditions the kidney still

TABLE 1

Determination of renin of systemic blood of anesthetized dogs after hemorrhage of 4 per cent of body weight

GROUP	DOG	WEIGHT	VOLUME OF PLASMA USED IN TESTING	UNITS OF RENIN IN VOLUME OF PLASMA USED IN TITRATION							
				Before	After (minutes)						
					30	40	50	60	70	90	120
		kgm.	cc.								
Normal dogs	1	21.0	8	0.00	0.46						
	2	13.0	8	0.00	0.00						
	3	12.5	8	0.00	0.00						
	4	11.3	8	0.00	0.00						
	5	8.5	12	0.00				0.41			
	6	10.0	12	0.00				0.11			
	7	11.5	12	0.00				0.54			
	8	10.0	6	0.00							0.00
	9	8.4	8	0.00		0.00					
	10	10.8	8	0.00						0.00	
	11	18.5	12	0.00				1.08		2.40	
	12	23.0	12	0.00				0.41			
	13	10.5	12	0.00			0.41				
	14	11.0	12	0.00					0.37		0.60
	15	12.6	12	0.00				1.20			
	16	11.0	12	0.00						0.92	
Nephrec- tomized dogs	17	13.3	12	0.07	0.03				0.00		
	18	10.9	12	0.04		0.00				0.00	
	19	18.0	12	0.00				0.00			
	20	16.0	12	0.00				0.00			
	21	9.7	12	0.04			0.01				
	22	8.9	12	0.02		0.03					

liberated renin on induction of hypotension with the same technique as in group I. Five dogs were injected subcutaneously with 10 mgm. per kilo of uranium nitrate and 3 dogs were given by stomach tube 10 to 25 mgm. per kilo of mercuric chloride. In both groups of animals widespread lesions of the tubular cells were observed such as granular degenerative lesions and necrosis of the cells, presence of hyaline cylinders in the lumen of the tubules, etc. The glomeruli showed only slight changes in some cases. Most of these dogs had low blood pressure before hypotension was induced by hemorrhage and in these cases renin was found in

the blood in varying amounts but its concentration increased after hemorrhage. When the blood pressure remained normal no renin or only small amounts were found before the induction of hypotension. The renin concentration increased very markedly after hemorrhage (table 2). The significance of these observations is still uncertain. The destruction of the tubules though extensive cannot be made complete; on the other hand the tubular lesions may facilitate the lib-
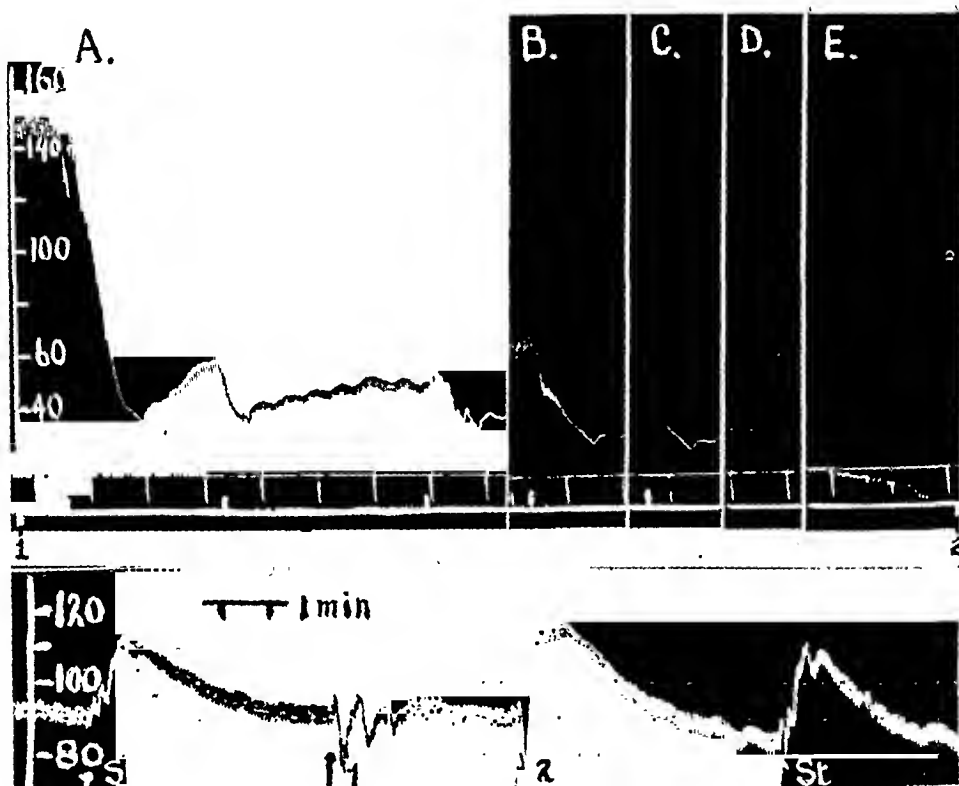
Fig. 1. *Above: Blood pressure of 13 kilo dog with denervated kidneys after hemorrhage.* Nembutal anesthesia. Carotid pressure in millimeters of mercury. Time in minutes. Upward signals indicate withdrawals of blood in order to maintain a low blood pressure. Downward signals at 1 and 2 indicate samples of blood withdrawn for estimation of renin before and 61 minutes after hemorrhage. Ten minute interval between A and B; 7 minutes between B and C; 20 minutes between C and D; and 7 minutes between D and E.

Below: Assay of renin in samples of blood obtained before and 61 minutes after hemorrhage of animal charted above. Dog of 8.5 kilos anesthetized with amytal. Carotid blood pressure in millimeters of mercury. *St* indicates intravenous injection of 1 unit of a standard solution of hypertensin; *1*, extract of blood plasma obtained before hemorrhage and incubated in the usual manner with hypertensinase-free precursor; *2*, extract of blood plasma obtained after 61 minutes of hypotension and treated in the same manner.

eration of renin into the blood assuming that this substance is produced by the tubular cells.

B. *Renal anoxia:* A possible explanation for the liberation of renin in hypotension would be anoxia of renal tissues due to the diminished blood flow through the organ. An attempt was made to produce renal anoxia without lowering blood pressure.

Group VI. To four anesthetized dogs receiving artificial respiration, potassium cyanide in doses of from 0.6 to 1.2 mgm. per kilo was given intravenously. Samples of femoral blood were taken before and at intervals after the administration of cyanide. No renin was found in the blood. That the ability of the renal cells to secrete renin was not suppressed by cyanide was shown by the
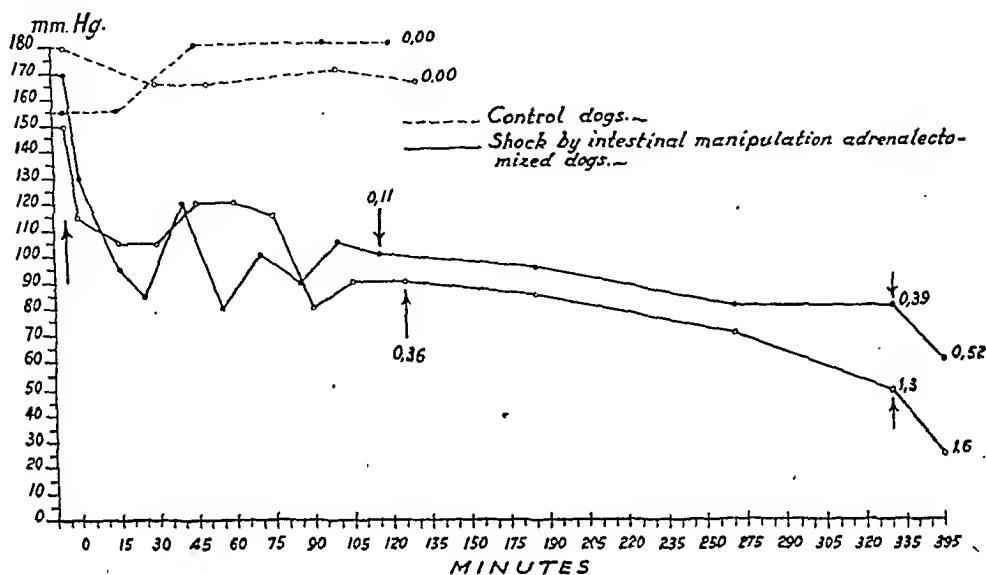
Fig. 2. Blood pressure and renin content of femoral blood of (a) control dogs and (b) adrenalectomized dogs in which shock was induced by intestinal manipulation.

TABLE 2

Determination of renin in systemic blood of dogs intoxicated with uranium nitrate and mercuric chloride before and after hemorrhage

DOG NO.	DRUG INJECTED AND DOSE	BLOOD PRESSURE BEFORE HEMORRHAGE	UNITS OF RENIN IN 12 CC. OF PLASMA USED IN TITRATION	
			Before hemorrhage	75 minutes after hemorrhage
		mm.Hg		
23	U.N. 10 mgm./kgm.	70	1.20	1.60
24	U.N. 10 mgm./kgm.	70	1.20	2.70
25	U.N. 10 mgm./kgm.	120	0.60	1.80
26	U.N. 10 mgm./kgm.	140	0.00	3.70
27	U.N. 10 mgm./kgm.	125	0.10	1.40
28	HgCl₂ 25 mgm./kgm.	90	0.03	0.57
29	HgCl₂ 20 mgm./kgm.	120	0.00	0.77
30	HgCl₂ 10 mgm./kgm.	105	0.70	1.80

appearance of large amounts of renin in the blood after hemorrhage was produced in the usual manner in these intoxicated dogs (fig. 3).

Group VII. In a large gasometer with a capacity of 100 liters, mixtures of respiratory gases were prepared and anesthetized dogs were artificially ventilated with these mixtures by means of a special pump (table 3). Dog 31 breathed a mixture with 8 per cent oxygen during one hour and no renin appeared in the

blood. Dogs 32 and 35 breathed a mixture with 6 to 7 per cent oxygen and no CO_2 for 50 and 30 minutes respectively after which no renin was found in the blood. In the latter dog an analysis of blood gases was made at the end of the
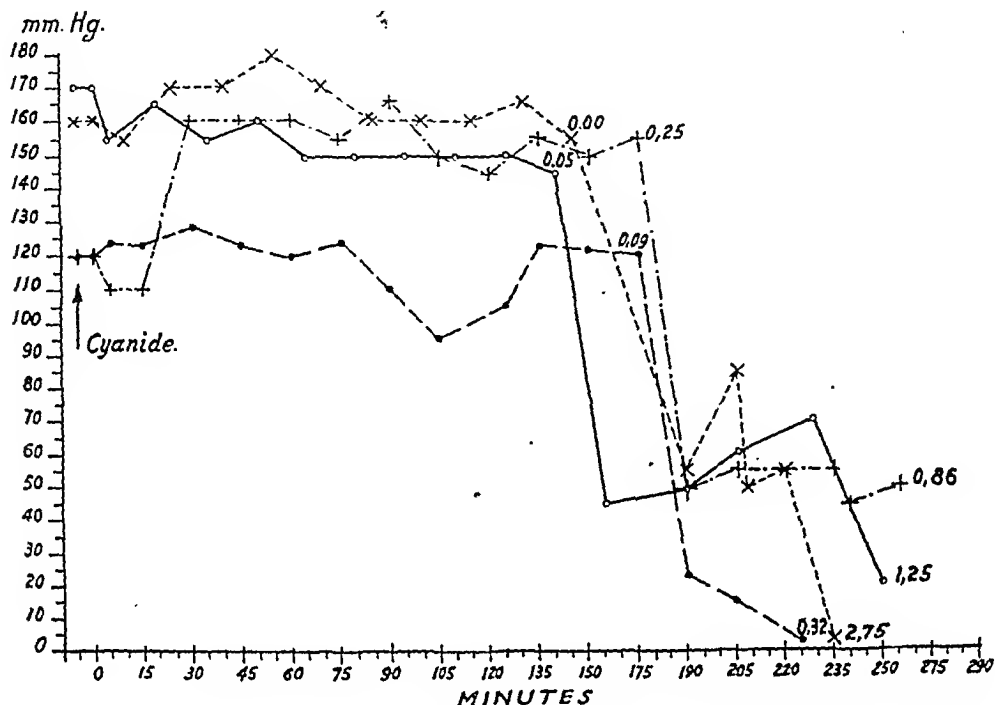
Fig. 3. Blood pressure and renin content of femoral blood of dogs injected with KCN and subsequently subjected to hemorrhage.

TABLE 3

Determination of renin in systemic blood of anesthetized dogs breathing mixtures of respiratory gases

DOG	WEIGHT	VOLUME OF PLASMA USED FOR TESTING	MIXTURE OF GASES BREATHED	UNITS OF RENIN IN VOLUME OF PLASMA USED FOR TITRATION							
				Before	After (minutes)						
					20	30	40	50	60	70	80
	kgm.	cc.									
31	7.5	12	8% O_2	0.00			0.00		0.00		
32	9.4	12	6 O_2	0.00	0.00			0.00			
33	8.2	12	8 O_2	0.05			0.83				
34	12.7	12	8 O_2	0.00					0.20		
35	10.0	12	6½ O_2	0.00		0.00				0.00	
36	9.8	12	49 O_2; 2%CO_2	0.02							0.21
37	11.0	12	7 O_2; 5 CO_2	0.00			0.00				
38	9.3	12	7 O_2; 4½ CO_2	0.00	0.00		0.00				

experiment and only 24 per cent saturation with O_2 was found in femoral blood. Dogs 37 and 38 breathed a mixture of 7 per cent oxygen and 4.5 to 5 per cent CO_2 for 38 and 40 minutes respectively after which no renin was found in the blood.

In two dogs, one after breathing a mixture of 8 per cent oxygen for 60 minutes and the other after breathing a mixture of 49 per cent oxygen and 2 per cent CO_2 for 80 minutes, 0.20 and 0.21 unit of renin respectively was found in the blood. In the first of these dogs the blood pressure had gradually fallen from 150 to 95 mm. Hg in the course of the experiment. In the second, the blood pressure was rather low to begin with (110 mm. Hg) and remained at approximately the same level throughout the experiment.
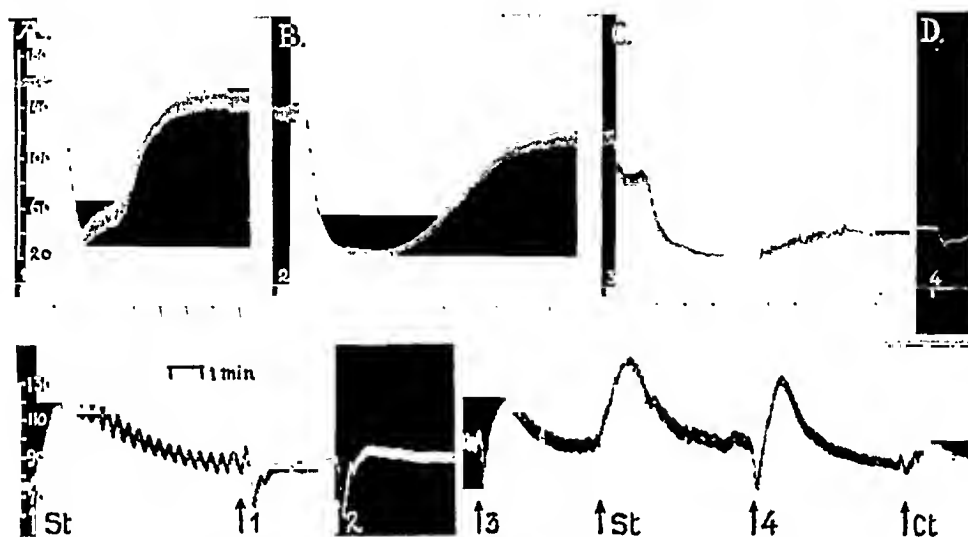
Fig. 4. *Above: Effect of repeated injections of amytal on blood pressure of 12 kilo dog anesthetized with nembutal.* Carotid blood pressure in millimeters of mercury. Figures indicate time at which the samples of blood were obtained for the determination of renin. Time in minutes. A, short and profound hypotension is produced by the intravenous injection of 0.15 gram amytal. Between A and B, 25 minute interval. B, longer and more pronounced hypotension produced by the intravenous injection of 0.10 gram of amytal. Between B and C, 4 minute interval. C, a new injection of 0.10 gram amytal produces a definitive lowering of blood pressure. Between C and D, 7 minute interval.

Below: Assay of renin in samples of blood obtained as indicated by the figures in the chart above. Dog of 8.0 kilos anesthetized with amytal. Carotid blood pressure in millimeters of mercury. The samples of plasma were incubated for 2 hours at 37°C. with 8 cc. of hypertensinase-free precursor. *St*, injection of 1 unit of a standard solution of hypertensin. *Ct*, injection of extract of control plasma.

To make sure that the ability of the renal cells to secrete renin was not altered during anoxemia, dog 33 breathing 8 per cent oxygen was subjected to hemorrhage and after 39 minutes 0.83 unit of renin was found in the systemic blood.

C. *Short periods of arterial hypotension:* To confirm our suspicion that some of the positive results obtained by the breathing of gas mixtures poor in oxygen or rich in CO_2 were due to the lowering of the blood pressure, short periods of hypotension were induced in 4 anesthetized dogs by the intravenous injection of amytal or by rapid removal of moderate amounts of blood. Short periods of profound hypotension of only 4 and 11 minutes in some cases (fig. 4) were sufficient to produce the liberation of renin by the kidney.

DISCUSSION. One important point must be kept in mind in interpreting the results of the experiments reported and that is that the accumulation of renin in the general blood in amounts sufficient to be detected by the method used means that the rate at which renin is liberated by the kidney is greater than the rate of its destruction or elimination by the body. In other words, the kidney may secrete renin into the blood in increased amounts and still if the rate of destruction of renin is also increased its presence in the systemic blood may not be detected.

The question of the concentration reached by renin in the blood is also of importance. When we say that no renin is present in the blood, we mean that no formation of hypertensin can be detected when a certain amount of plasma (6 to 12 cc.) is incubated with hypertensin precursor during 2 hours at 37°C. It is possible that by using larger amounts of plasma or longer periods of incubation, the presence of renin could be detected. Unfortunately in the method of Leloir et al. (10) for the estimation of renin, the amount of plasma which can be incubated is limited by the toxicity of plasma extracts.

The volume of circulating blood may also be of importance in determining the concentration of renin in the blood. It is conceivable that the smaller the blood volume the more rapidly will renin accumulate in the blood.

For all these reasons we cannot affirm with certainty that anoxemia of the kidney tissue is not the primary factor in the secretion of renin but our experiments are in favor of this view. In fact, discarding those in which the blood pressure was low, in none of the experiments in which severe anoxemia or histotoxic anoxia was produced could renin be detected in the general blood.

The blood pressure seems more likely to be the stimulus for the secretion of renin by the kidney, but we do not know in what way this stimulus acts upon the kidney. Assuming that the tubular cells are the site of formation of renin, it is possible that a reduced pressure in the capillaries which irrigate them alters in some manner the normal function of the cell or its permeability. These hypotheses remain to be proven. One firm conclusion, however, can be drawn from this study, i.e., that hypotension, even of short duration, causes the intact normal kidney to secrete renin into the blood in amounts sufficient to be estimated in the general blood of normal anesthetized dogs.

The normal kidney thus seems to participate in the regulation of the blood pressure. A lowering of the blood pressure determines the internal secretion of renin through which the formation of hypertensin will contribute to the normalization of the blood pressure. Renin appears to be a substance which not only appears as the result of a pathological disturbance of the kidney, but one of the many physiological substances which the body uses for its homeostasis at least in cases of emergency.

SUMMARY

Profound lowering of the blood pressure by hemorrhage (4 per cent of body weight) or shock causes the liberation of renin by the intact kidney of normal anesthetized dogs. Renin can be detected in the systemic blood of these dogs.

Renin could not be detected in the blood of nephrectomized dogs after hemorrhage or in normal dogs intoxicated with KCN or subjected to respiration of mixtures poor in oxygen.   After short periods of 4 to 11 minutes of profound arterial hypotension renin could be detected in the systemic blood of normal dogs.

The inference is drawn that the kidney participates in the regulation of arterial blood pressure.   When the blood pressure decreases the normal kidney secretes renin which through the formation of hypertensin tends to the restoration of normal blood pressure.   Renin appears to be a substance which the body uses to maintain homeostasis.

REFERENCES

(1) BRAUN-MENENDEZ, E., J. C. FASCIOLO, L. F. LELOIR AND J. M. MUÑOZ.   (a) Rev. Soc. Argentina Biol. **15**: 420, 1939.
(b) Compt. Rend. Soc. Biol. **133**: 731, 1940.

(2) BRAUN-MENENDEZ, E., J. C. FASCIOLO, L. F. LELOIR AND J. M. MUÑOZ.   J. Physiol. **98**: 283, 1940.

(3) DELL 'ORO, R. AND E. BRAUN-MENENDEZ.   To be published.

(4) FASCIOLO, J. C., B. A. HOUSSAY AND A. C. TAQUINI.   J. Physiol. **94**: 281, 1938.

(5) FREEDMAN, A.   Am. Heart J. **20**: 304, 1940.

(6) GOVAERTS, P.   Bull. Acad. Méd. Belg., p. 357 (Sept.) 1939.

(7) HOUSSAY, B. A., E. BRAUN-MENENDEZ AND L. DEXTER.   Ann. Int. Med., in press.

(8) HOUSSAY, B. A. AND L. DEXTER.   Ann. Int. Med., in press.

(9) HOUSSAY, B. A. AND J. C. FASCIOLO.   (a) Rev. Soc. Argentina Biol. **13**: 284, 1937.
(b) Compt. Rend. Soc. Biol. **127**: 147, 1938.

(10) LELOIR, L. F., J. M. MUÑOZ, E. BRAUN-MENENDEZ AND J. C. FASCIOLO.   Rev. Soc. Argentina Biol. **16**: 635, 1940.

(11) MERRILL, A., J. R. WILLIAMS AND T. R. HARRISON.   Am. J. Med. Sc. **196**: 18, 1938.

(12) MUÑOZ, J. M., E. BRAUN-MENENDEZ, J. C. FASCIOLO AND L. F. LELOIR.   Nature **144**: 980, 1939.

(13) MUÑOZ, J. M., E. BRAUN-MENENDEZ, J. C. FASCIOLO AND L. F. LELOIR.   Am. J. M. Sc. **200**: 608, 1940.

(14) PAGE, I. H.   This Journal **130**: 29, 1940.

(15) PAGE, I. H. AND O. M. HELMER.   J. Exper. Med. **71**: 495, 1940.

(16) RODBARD, S.   This Journal **133**: P429, 1941.

(17) TIGERSTEDT, R. AND P. G. BERGMANN.   Skand. Arch. f. Physiol. **8**: 223, 1898.

(18) WAKERLIN, G. E. AND G. R. CHOBOT.   Proc. Soc. Exper. Biol. and Med. **40**: 331, 1939.

THE EFFECT OF METHEMOGLOBIN ON THE EQUILIBRIUM BETWEEN OXYGEN AND HEMOGLOBIN

ROBERT C. DARLING AND F. J. W. ROUGHTON

From Fatigue Laboratory, Harvard University, Boston, Mass.

Methemoglobin may be formed from hemoglobin in vitro by a wide range of oxidizing agents. Such compounds also act in vivo if they can permeate the red cell membrane; other substances such as aniline and its derivatives (including the sulfanilimide series) are ineffective with hemoglobin solutions but do form methemoglobin in vivo, possibly because they give rise, in the course of their metabolism, to intermediate compounds with oxidizing properties, e.g., aminophenol or phenylhydroxylamine. Methemoglobinemia is accordingly a fairly common condition both in chemical industries and in drug therapy (for reviews v. Peters and Van Slyke, 19; Wendel, 22). The symptoms are very similar to those produced by an equivalent degree of CO-hemoglobinemia, (see especially (14)) the toxic effects in both cases being much greater than those produced by the same amount of anemia. Some further action besides the lowering of the O_2 carrying power of the blood is therefore indicated. In the case of methemoglobinemia this has been thought to be due entirely to the direct effect of the methemoglobin producing agent on the tissues, e.g., vasodilatation in the case of nitrites. Carbon monoxide, however, does not act directly on the tissues except at pressures much higher than those usually found in CO poisoning, and its extra effect has been explained in another way. Douglas, Haldane and Haldane (12) (see also Stadie and Martin, 20) observed that when part of the hemoglobin in blood is combined with CO, the oxygen dissociation curve of the remaining hemoglobin is shifted to the left and is less S-shaped. This "affinity effect" greatly hinders the unloading of O_2 from the blood to the tissues and in Haldane's view (13) accounts for the fact that "miners may be doing their ordinary work though their hemoglobin percentage is reduced to half or less by ankylostomiasis . . . whereas a person whose blood is half saturated with CO is practically helpless."

These findings with CO hemoglobin made us ask whether methemoglobin might not have a similar effect on the dissociation curve of oxyhemoglobin. From a theoretical standpoint this is a reasonable question, since methemoglobin has long since ceased to be regarded as an irreversible compound: Conant and his colleagues (7, 8) have indeed shown that the same equilibrium point is reached between CO-hemoglobin, methemoglobin, CO, ferricyanide and ferrocyanide from whichever side the equilibrium is approached. A similar reversible equilibrium was also demonstrated in the presence of O_2 in place of CO. Now since Haldane's work has proved that in the reversible system CO hemoglobin-oxyhemoglobin-hemoglobin-CO-O_2, the presence of CO and COHb affects the equilibrium between O_2 and hemoglobin (and similarly the presence of O_2

and O_2Hb affects the equilibrium between CO and hemoglobin) it seems rational by analogy to expect that in the reversible system oxyhemoglobin-hemoglobin-methemoglobin-ferricyanide-ferrocyanide-O_2 the presence of ferricyanide and methemoglobin would affect the equilibrium between O_2 and hemoglobin. In the experiments of Conant and colleagues the concentrations of ferricyanide and of CO (or O_2) were too high for there to be appreciable amount of reduced hemoglobin present; a search through the rest of the literature has equally failed to reveal any systematic data of the effect of methemoglobin on the oxyhemoglobin dissociation curve. In view of the theoretical interest of the matter and also of its possible clinical, industrial and even military importance we therefore decided to determine the effect of graded amounts of methemoglobin, made in several ways, upon the oxyhemoglobin dissociation curve. To avoid uncertainties due to pH and other ionic effects we have worked mainly with solutions of washed red cells in 0.6 M phosphate buffer. In this medium the dissociation curve in absence of methemoglobin is found to be practically the same as in whole blood in which the pH of the red cells is brought to the same value by CO_2 instead of phosphate. The progressive shift of the dissociation curve with increasing methemoglobin which we report below might thus be reasonably expected to occur under more physiological conditions. This we have confirmed by finding that the same shifts take place when methemoglobin is formed in the circulating blood after subcutaneous injection of sodium nitrite, but that the dissociation curve returns to normal when the methemoglobin has had time to be reduced back again to ordinary hemoglobin by the natural reducing systems of the red cell. These results, with some theoretical and practical implications, are presented below.

METHODS AND PROCEDURES. We have used three methods for making methemoglobin from hemoglobin in vitro:

1. Addition of potassium ferricyanide
2. Incubation at pH 5.6 with low O_2 pressures (5)
3. Addition of sodium nitrite

Of these the first has been most fully investigated and is the best understood. Accordingly we have done most of our work with ferricyanide and have used the other methods for special conditions and for elimination of effects specific to ferricyanide. We have confirmed previous findings that 1 molecule of $K_3Fe(CN)_6$ per atom of Fe in hemoglobin forms methemoglobin almost stoichiometrically. The excess ferricyanide required to convert hemoglobin to methemoglobin, even when solutions were in equilibrium with 150 mm. Hg pressure of oxygen, was found to be only 5 per cent on the average.

The preparation of the hemoglobin solutions and of the phosphate buffers, as also the technique for determining the dissociation curves, were the same in the ferricyanide experiments as in all the other experiments. Details as to these common features are given in this section, whereas details special to each of the three methods of making methemoglobin are given at the outset of the description of corresponding results.

Preparation of hemoglobin solutions. Fresh ox blood was obtained from a

slaughter house and defibrinated immediately by shaking with glass beads. Human red cells were furnished through the courtesy of Dr. R. M. Ferry; they were the unused by-product in the collection and concentration of serum proteins. Both lots of red cells were centrifuged and washed with 1.5 per cent NaCl three times, minimal hemolysis was observed in the wash saline but the red cell mass obviously shrank under the action of the mildly hypertonic saline. Red cells after washing were frozen and stored in that state. Small lots of these were thawed freshly for each experiment. Generally the single freezing was sufficient for laking; on a few occasions repeated freezing was carried out to ensure laking.

In preparing the final dilutions of hemoglobin the recently thawed cells were mixed with about $\frac{1}{2}$ their volume of distilled water (to reduce the high viscosity) and centrifuged to remove the red cell ghosts. The supernatant liquid was perfectly clear. The oxygen capacity, after the addition of the buffers and other solution, was usually 10 to 12 vols. per cent.

Phosphate solutions. Three concentrated buffers of approximately pH 5.6, 6.8 and 7.4 were made up.

$$\text{pH } 5.6 = 1.009 \text{ M } KH_2PO_4 + 0.191 \text{ M } K_2HPO_4$$
$$\text{pH } 6.8 = 0.60 \text{ M } KH_2PO_4 + 0.60 \text{ M } K_2HPO_4$$
$$\text{pH } 7.4 = 0.24 \text{ M } KH_2PO_4 + 0.96 \text{ M } K_2HPO_4$$
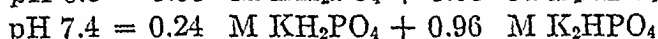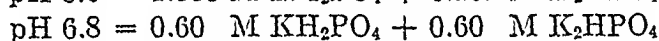
The potassium salts were used in preference to sodium on account of their higher solubility. CO_2 was removed from each buffer by evacuating and shaking in a large bottle three times.

Determination of the oxyhemoglobin dissociation curve. The technique was essentially that of Dill, Hurtado, Graybiel and Tacquini (11) except that the gas phase in the tonometer was almost CO_2-free (analysis usually showed less than 0.1 per cent). Barcroft type tonometers of about 300 cc. capacity were flushed with N_2 and appropriate amounts of O_2 were added from a gas burette. Four or 5 cc. of hemoglobin solution were added to each, the taps sealed with mercury and equilibration carried out for 15 minutes at 37° in a water bath. The solutions were then removed under water into small sampling tubes over mercury where they were stored in the ice box until analyzed. The gas phase of the tonometers was analyzed after they had cooled to room temperature. Gas pressure in the tonometer, temperature and barometric pressure were measured at the time of taking the sample into the Haldane apparatus. From these the pO_2 in the tonometer at 37° during equilibration was calculated. The hemoglobin solutions were analyzed for O_2 from 1 or 2 cc. samples in the Van Slyke apparatus. Dissolved oxygen in the buffered mixture was calculated from Brooks' data, converting to the higher temperature of 37°. The factor at 37° was taken as 0.0025 vol. per cent per mm. Hg of pO_2. The oxygen capacity of each sample was determined by equilibrating the residual sample after the analysis for content at room temperature in an open flask. At this temperature (25°C) the oxygen solubility factor of 0.0028 was used.

RESULTS. *Experiments with methemoglobin made by ferricyanide.* Each solution on which an oxyhemoglobin dissociation curve was obtained consisted

of: 5 parts thawed red cells; 10 parts 1.2 M phosphate buffer; 2 parts $K_3Fe(CN)_6$ solution; 3 parts distilled water.

The ferricyanide solution was prepared from stock 0.02 M $K_3Fe(CN)_6$ made from C. P. chemicals, and diluted to the strength calculated to give the methemoglobin concentration desired.

Oxyhemoglobin dissociation curves on such mixtures of hemoglobin and methemoglobin in 0.6 M phosphate, pH 7.4, are presented in figure 1 for the ox blood and figure 2 for the human. The progressive shift to the left with increasing methemoglobin and the fading out of the S-shaped inflection are at once apparent. Since ferricyanide is the other product formed when ferricyanide reacts
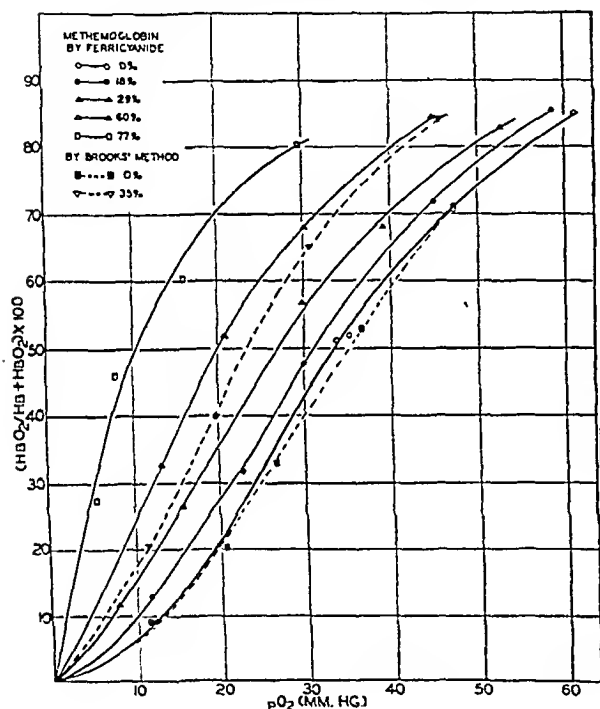
Fig. 1
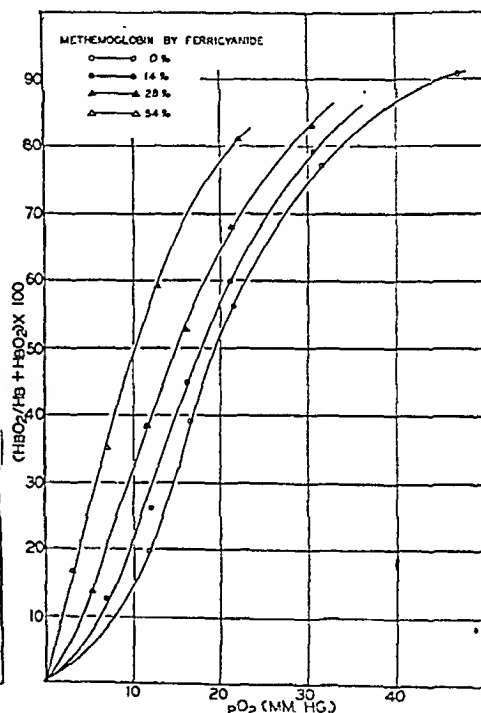
Fig. 2

with hemoglobin to form methemoglobin, a control curve was obtained at a ferrocyanide concentration equal to the ferricyanide concentration used. This control curve agreed within experimental error with that obtained without added ferri- or ferrocyanide. The shift of the dissociation curve must then be seemingly due to the influence of the methemoglobin and not to the inorganic ions.

Qualitatively the changes are similar to the effects of COHb but roughly twice as much MetHb as COHb is required to produce a given shift (see also fig. 4). The same relative shifts in the curve were found in determinations at (1) pH 6.8, 37°C (2) pH 7.4, 25°C. The effect of methemoglobin formed by ferricyanide is thus independent of species, temperature and pH within the limits tested.

Experiments with methemoglobin made by aerobic oxidation. Brooks (5) has

shown that methemoglobin is formed at a unimolecular rate, when hemoglobin is equilibrated for many hours with a gas phase containing O_2, the velocity constant depending on the O_2 pressure, pH and temperature. His work provided us with an entirely independent method of making methemoglobin, by means of which we hoped to remove any doubts still remaining that the shift of the dissociation curve might be in some way due to the ferricyanide in spite of the controls already mentioned. For making methemoglobin by Brooks' method, the hemoglobin was made up in the pH 5.6 phosphate buffer exactly as before but omitting the ferricyanide. One half of this lot was equilibrated in a tonometer at 37° for 2 hours, at the end of which time its O_2 capacity had diminished by 35 per cent and, according to Brooks, an equivalent amount of methemoglobin had been formed. Both this sample and that not incubated were adjusted approximately to pH 7.4 by adding 1 cc. of 1.923 M K_2CO_3 to each 10 cc. of hemoglobin solution, and then evacuating and shaking for five periods of three minutes to remove CO_2.

The dissociation curves of these two solutions are shown by dotted lines in figure 1. The same type of shift is again apparent: in the lower half of the curve the shift is the same as with 35 per cent methemoglobin made by ferricyanide, but in the upper half of the curve a greater shift is observed. The reason for this discrepancy is not clear, but it may be that the incubation at pH 5.6 also caused incipient changes in the globin thus changing slightly the characteristics of the curve. After 24 hours' incubation at pH 5.6 an obvious change has been found to occur (v. Discussion). We have not searched for an exact explanation, since the discrepancy was unimportant for our purpose, which was to demonstrate qualitatively the shift due to methemoglobin in the absence of a chemical oxidizing agent such as ferricyanide.

Methemoglobin forms slowly from oxygenated hemoglobin solutions or red cells even when they are kept frozen in the solid state. The chemical mechanism of the change is probably the same as in Brooks' experiments. It was therefore of some interest to find that hemoglobin solutions prepared from the stock of human red cells, after 3 months' storage at $-5°C$, contained about 12 per cent methemoglobin and 5 per cent inactive hemoglobin, and that the dissociation curve was shifted to the left by an amount agreeing with that to be expected from the results obtained with methemoglobin prepared by Brooks' method.

In vivo experiments with nitrite methemoglobin. The action of nitrite on hemoglobin is extremely complicated. It varies with the molecular ratio of nitrite to hemoglobin, pH, presence or absence of O_2 and reducing agents, and possibly with other factors. Amongst the products of reaction found under varying conditions in vitro are methemoglobin, NO-hemoglobin and NO-methemoglobin (3, 4, 6, 14, 15, 16, 17). There seems, however, fairly general agreement that when nitrite is injected intravenously or subcutaneously methemoglobin alone is produced in the red cells up to a maximum value and then as Haldane, Makgill and Mavrogordato (14) were the first to show, the normal hemoglobin is gradually restored. With intravenous injection of 30 mgm. nitrite per kgm. body weight, Wendel (23) found that methemoglobin rapidly accumulates in the

red cells and after 1–2 hours reaches a maximum corresponding to a loss of about $\frac{2}{3}$ of the oxygen capacity. It then progressively disappears, owing to the enzyme systems of the red cells reducing it back to ordinary hemoglobin. The reducing substances concerned may be glucose and/or lactate (24). The reversion is complete after 8 to 9 hours; reversal at a similar rate is found if the red cells are withdrawn at the peak of the methemoglobinemia and incubated in vitro at 37°C. These observations afforded us a ready opportunity to test two important questions: 1. Does methemoglobin produced in vivo shift the oxygen hemoglobin dissociation curve just as it does when produced in vitro? 2. When the methemoglobin is reduced back again to normal hemoglobin does the dissociation curve revert to its original position?

The following is a protocol of an experiment on a 17 gram dog to answer these two questions.

9:15 a.m.   Arterial blood obtained. Hematocrit = 45.3; HbO_2 cap. = 20.6 vol. per cent.

9:24 a.m.   15 cc. 5 per cent $NaNO_2$ injected subcutaneously.

9:24–11:30   Slight cyanosis of tongue appeared. Dog active, apparently unaffected.

11:30 a.m.   20 cc. 5 per cent $NaNO_2$ injected subcutaneously.

12:15 p.m.   Vomited. More cyanotic.

12:50 p.m.   Abnormally quiet. Obviously ill.

1:00 p.m.   Vomited again. Scarcely able to stand.

1:20 p.m.   Arterial puncture unsuccessful. 25 cc. venous blood obtained deep chocolate in color. Hematocrit 46.4; HbO_2 cap. = 6.5 vol. per cent.

2:30 p.m.   Unable to stand, respirations gasping. O_2 administration begun and continued intermittently until 4:30 p.m. Oxygen caused prompt improvement in color, return of normal respirations and increased strength. Dog was able to stand after 15 min. of O_2. Stopping oxygen caused return of stupor and collapse.

4:30 p.m.   Finally awake but very weak without O_2. Still very cyanotic.

8:00 a.m.   Next morning. Appeared normal, active, not cyanotic. 17 cc. venous blood taken, normal color. Hematocrit 46.8 HbO_2 cap. = 21.5 vol. per cent.

Next afternoon: The remainder of the blood, drawn at the height of the methemoglobinemia was found, after 38 hours in the ice chest, to contain only reduced hemoglobin. The O_2 content was practically nil and the O_2 capacity had returned to 20 vol. per cent.

The train of symptoms through which the dog passed, and the changes in the hemoglobin of his red cells during the course of the experiment are in full accord with the observations of previous workers.

The oxyhemoglobin dissociation curves on the blood samples drawn before, at the height, and at the end of the methemoglobinemia are shown in figure 3. Equilibration was carried out at a CO_2 pressure of 40 mm. Hg, and the blood samples were analyzed promptly before any reduction of methemoglobin could occur. Again there appears the striking shift and change in the shape of the curve in the presence of methemoglobin. The blood 23 hours after first giving nitrite has however exactly the normal properties and the normal dissociation curve. Since no evidence was found of pigments in the plasma of any of the blood samples, it is inconceivable that new hemoglobin equivalent to two-thirds

the total hemoglobin of the blood could be formed in so short a time as one day. The original hemoglobin of the blood in vivo must therefore have been largely changed to methemoglobin and then returned to normal again. In so doing, the dissociation curve of the oxyhemoglobin suffered the characteristic shift in presence of methemoglobin, but reverted to normal with the reduction of the

DOG BLOOD IN NITRITE POISONING AT $pCO_2=40$ MM. HG.

o———o BEFORE NITRITE
△———△ 5 HOURS AFTER NITRITE - 69% METHEMOGLOBIN
●———● 23 HOURS AFTER NITRITE - 0% METHEMOGLOBIN

$\dfrac{HBO_2}{HB + HBO_2} \times 100$

pO_2 (MM. HG.)
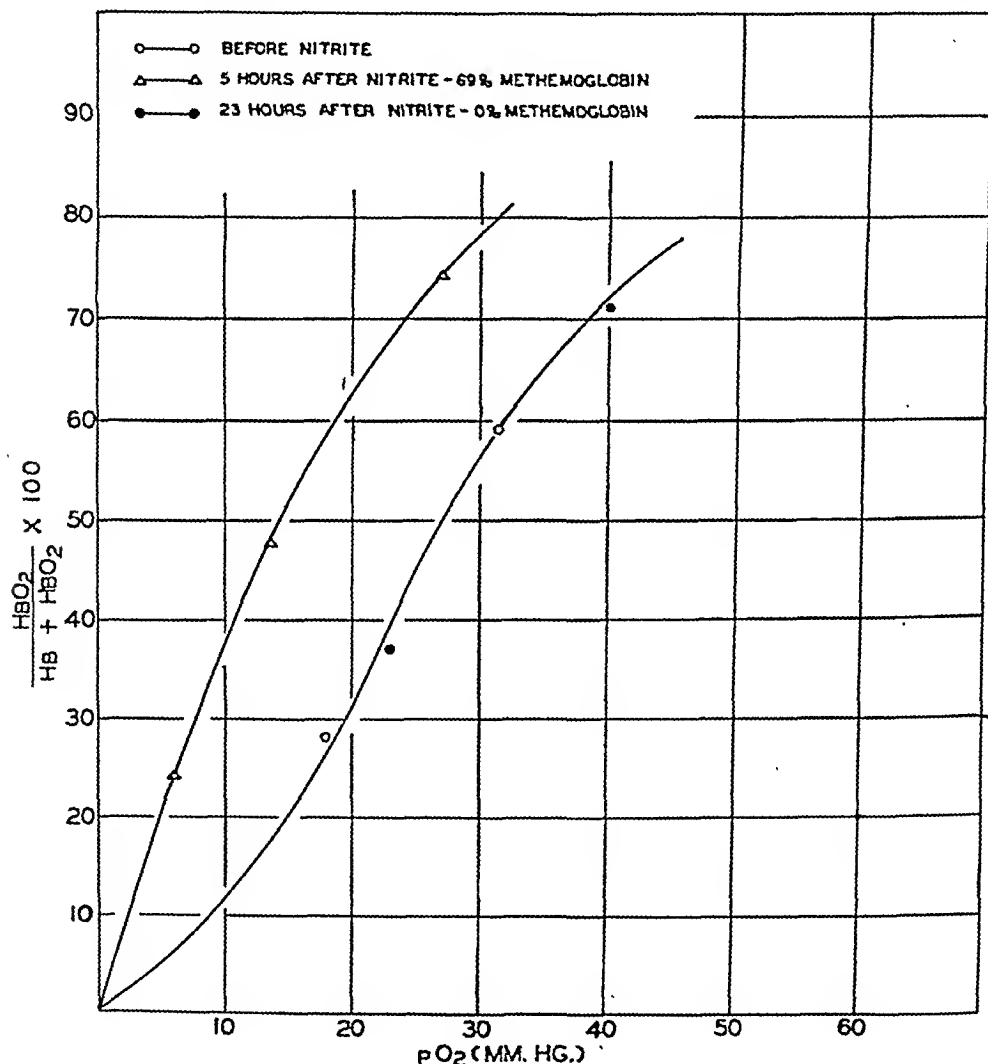
Fig. 3

methemoglobin. We accordingly conclude that the methemoglobin shift of the dissociation curve occurs in vivo and is "reversible."

In vitro experiments with nitrite. One of the curves of figure 4b (solid tri-angles) shows the oxyhemoglobin dissociation curve of a hemoglobin solution in 0.6 Molar phosphate buffer at pH 7.4 to which $NaNO_2$ had been added to a con-centration of 0.0014 M. The technique of preparing the solution was similar

to that used with ferricyanide. The exact nature of the pigments was not determined; the gross color was that of methemoglobin. The oxygen capacity was reduced by 56 per cent and the curve shows a shift to the left in the same range as that produced by 56 per cent methemoglobin formed by ferricyanide.

An attempt was made to obtain a dissociation curve on whole heparinized human blood to which $\frac{1}{20}$ its volume of 0.055 M $NaNO_2$ had been added in vitro. An exact curve was impossible to obtain since it was found that the oxygen capacity gradually fell from 20 vol. per cent to 11.5 then rose over several hours to 17.4. Furthermore it was found that the drop in O_2 capacity was not identical
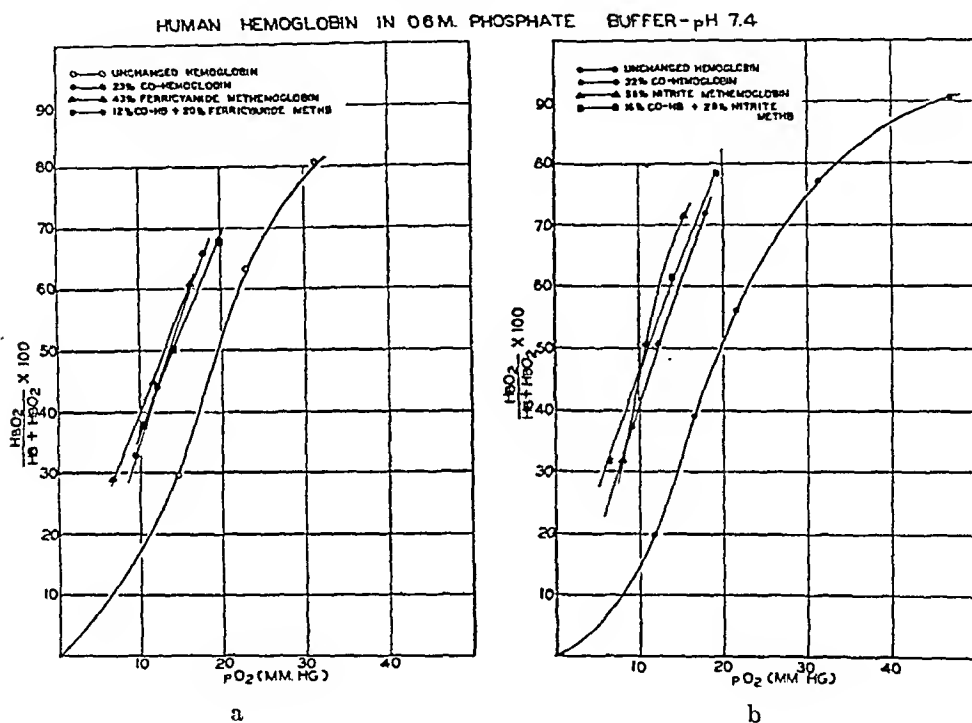
Fig. 4

at different O_2 pressures and temperatures. It can be concluded however even from this inexact data that the curve was shifted to the left.

Additive effect of CO and methemoglobin. The qualitative similarity in the effects of COHb and MetHb on the oxyhemoglobin dissociation curve suggests that a similar mechanism must exist in the two cases. If so, the effects of the two compounds might well be additive when both are simultaneously present. This matter is also of interest to test from the practical angle in view of statements that 1, CO and nitrous fumes are synergistic in their action, a mixture of the two gases being more toxic than either one when breathed alone. Individuals are sometimes exposed simultaneously to these two gases. 2, the toxic action of nitrous fumes may in some cases be in part due to methemoglobin formation (for discussion, see 17).

Figure 4a shows the effects of *a*, 43 per cent MetHb made by ferricyanide;

b, 23 per cent COHb; *c*, a mixture containing 12 per cent COHb and 20 per cent MetHb on the oxyhemoglobin dissociation curve in 0.6 M phosphate, pH 7.4, 37°C. The 43 per cent MetHb solution and curve were obtained as above. In the case of *b* and *c* a portion of the O_2Hb solution used in the control curve was converted entirely into COHb by equilibration with 7 per cent CO for 30 minutes. For the final mixtures, 23 per cent and 12 per cent of this solution were used: the remainder was unchanged O_2Hb in *b* and unchanged O_2Hb plus K_3Fe$(CN)_6$ to a concentration of 0.0013 M in *c*. Thirty cubic centimeters of the solutions *b* and *c* were used in 300 cc. tonometers, so that the loss of CO to the gas phase should be insignificant, and the per cent COHb in solution remain constant. This was confirmed by actual analyses of the hemoglobin solution after 1 to 2 hours' equilibration.

Figure 4b shows similarly the effects of *d*, 56 per cent nitrite MetHb; *e*, 32 per cent COHb; *f*, a mixture of 29 per cent nitrite MetHb + 16 per cent COHb. The solutions were prepared as in the experiments of figure 4a save that there was a final concentration of $NaNO_2$ in *d* of 0.0014 M and in *f* of 0.0007 M in place of ferricyanide.

Inspection of figure 4a, b, shows close agreement between the curves in presence of MetHb, COHb, and 50:50 mixtures of the two, both in the case of the methemoglobin made from ferricyanide and of "nitrite" methemoglobin. The effects of methemoglobin and CO hemoglobin are thus clearly additive.

The quantitative shifts produced by COHb in figures 4a and 4b agree with those found by Douglas, Haldane and Haldane and are greater than would be expected from the work of Stadie and Martin.

DISCUSSION. The results reported in this paper are worthy of discussion both from the clinical and theoretical viewpoints.

Clinical significance. The definite and reversible shift of the dissociation curve to the left in presence of methemoglobin constitutes, we believe, the first definite proof that presence of methemoglobin in the red cells has an adverse effect on the respiratory function of the blood besides the loss of O_2 carrying capacity which methemoglobin formation entails. This new effect of methemoglobin helps to explain the close resemblance mentioned in the introduction between the toxic effects of equal degrees of CO-hemoglobinemia and methemoglobinemia. It suggests that more weight should be given than hitherto to the appearance of methemoglobin in the blood either in poisoning or in drug therapy. Although 20 to 30 per cent methemoglobin should be tolerated well by normal individuals, just as is 20 to 30 per cent CO hemoglobin, more than that would be likely to lead to serious tissue anoxemia quite independently of any direct toxic action of the methemoglobin-producing agent on the tissues. In ill subjects a danger point might well be reached at lower concentrations of methemoglobin, and continued use of the offending drug in the face of mounting methemoglobinemia would only be wise if the drug was for other reasons absolutely essential.

The treatment for methemoglobinemia recommended by Wendel (23) is the administration of methylene blue, which catalyzes the enzyme systems of the red cell normally responsible for the reduction of methemoglobin in vivo. He

has shown that with intravenous injection of 2 mgm. methylene blue per kilogram weight, the rate of restoration to ordinary hemoglobin is accelerated about fourfold in dogs. Our studies lend point to the further development of such work.

The synergistic effect of CO and nitrous fumes, referred to previously, would be readily explained by the additive effect shown in figure 4b, if methemoglobin as well as CO hemoglobin, were certainly formed in the red cells under such circumstances. According to von Oettinger's recent review (17), however, the incidence of methemoglobinemia in nitrous fume poisoning is spasmodic and in general far less common than other symptoms, e.g., especially irritation of the respiratory passages and pulmonary edema.

Methemoglobin is probably produced more readily by the NO than by the NO_2 present in the nitrous fumes: the latter is naturally apt to be present in excess of the former and at concentrations of 50 to 100 parts per million (NO_2) produces pulmonary irritation within $\frac{1}{2}$ hour. If a man breathed 75 parts per million nitrous fumes for 1 hour and were to absorb the whole of the fumes into his blood, calculation shows that the oxygen capacity of his blood would only be reduced by 4 per cent assuming a ventilation rate of 10 liters per minute, a blood volume of 5 liters and the development of an equivalent of methemoglobin for every molecule of nitrogen oxide absorbed. The natural reducing systems of the red cell are capable, according to Wendel (23), of reducing about 10 per cent of the total hemoglobin of the blood from methemoglobin per hour. It is thus not surprising that in the majority of cases of nitrous fume poisoning, methemoglobinemia is negligible in comparison with pulmonary and other symptoms.

In case of methemoglobin-producing agents like aniline, which is a frequent source of methemoglobinemia amongst industrial workers, it has been observed that the condition appears much more gradually than after nitrite, and likewise disappears more gradually. Aniline itself is probably not the true oxidizing agent, but gives rise to the latter slowly; furthermore, the aniline itself may only be slowly eliminated from the body. In such case the reversibility within the body is much less apparent than with nitrite, and therapeutic measures should be devised to promote excretion or destruction of the noxious agent, as well as reduction of the methemoglobin.

Theoretical interpretation and suggestions. The S-shape of the oxyhemoglobin dissociation curve is now generally explained by the intermediate compound hypothesis which was first put forward in general form by Adair (1, 2) and was subsequently developed in a special form by Pauling (18, 10). According to this hypothesis the blood hemoglobin molecule, since it contains 4 ferrous atoms of iron, reacts with O_2 in four stages.

$$Hb_4 + O_2 \quad = Hb_4O_2 \tag{1}$$
$$Hb_4O_2 + O_2 = Hb_4O_4 \tag{2}$$
$$Hb_4O_4 + O_2 = Hb_4O_6 \tag{3}$$
$$Hb_4O_6 + O_2 = Hb_4O_8 \tag{4}$$
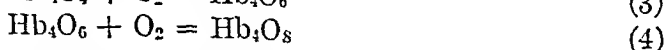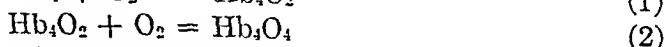
At very low O_2 pressures the principal oxygen compound formed must be the monoxy compound Hb_4O_2, and the dissociation curve is practically a straight

line: it soon, however, takes an upward turn, which is assumed to be due to the greater ease of formation of the dioxy compound, Hb_4O_4, once an appreciable amount of the monoxy compound has been formed. The Hb_4O_4 in turn increases the ease of formation of Hb_4O_6, etc. The S-shape of the CO-hemoglobin dissociation curve is similarly interpreted. To explain the effect of COHb in increasing the affinity of the residual hemoglobin for O_2 (fig. 4a and 4b) we must postulate that the formation of the monocarbonyl compound Hb_4CO, etc., not only increases the ease of attachment of further CO molecules, but also increases the ease of attachment of O_2 molecules to the residual iron atoms.

According to current conceptions, the iron atoms are solely ferric in the methemoglobin molecule, which may be written $(Fe^{+++})_4X$ in distinction from reduced hemoglobin, in which the iron atoms are ferrous $((Fe^{++})_4X)$.

Application of the intermediate compound hypothesis to the hemoglobin-methemglobin equilibrium leads to the postulation of intermediate compounds $(Fe^{++})_3 (Fe^{+++})_1X$, $(Fe^{++})_2 (Fe^{+++})_2X$, $(Fe^{++}) (Fe^{+++})_3X$. Since methemoglobin has been found to produce qualitatively the same shift in the oxyhemoglobin dissociation curve as CO-hemoglobin does, we conclude that the oxidation of one or more of the ferrous atoms to ferric must increase the affinity of the remaining ferrous atoms for O_2, in a similar but lesser degree than the effect produced by the combination of the same number of ferrous atoms with CO. This then is our interpretation of the results summarized in figures 1 and 2.

Two special cases can be conceived in which wholly ferrous and wholly ferric hemoglobin could coexist without mixed ferrous-ferric intermediates being present.

A. If the hemoglobin molecule contains not four but only one atom of iron, as in muscle hemoglobin and chironomus hemoglobin there can be no compounds of intermediate structure. Conversion of part of the hemoglobin to methemoglobin should not in these cases shift the dissociation curve from its normal position or shape which is that of a rectangular hyperbola. It would be interesting to test this deduction experimentally, and likewise to study the redox potentials of these special hemoglobins,[1] since the intermediate compound hypothesis has also been considered in the interpretation of the redox potentials of the hemoglobin-methemoglobin system in the case of mammalian blood hemoglobin (9, 21).

B. If two parts of normal hemoglobin solution $((Fe^{++})_4X)$ are quickly mixed with one part of hemoglobin solution which has been completely converted to methemoglobin $((Fe^{+++})_4X)$, the solution just after mixture should contain only completely ferrous and ferric molecules. If the dissociation curve could be obtained quickly enough there should be either no shift or a much smaller shift than usually found with 33 per cent MetHb. We did one test of this kind at pH 7.4 with methemoglobin made by ferricyanide, but we could not reduce the time of the experiment below 15 minutes, and it is perhaps not surprising that

[1] Taylor, J. F. and Morgan, V. E. J. Biol. Chem. 144: 15, 1942 have just reported a study of the redox potential of the metmyoglobin-myoglobin system which indicates a simple unimolecular reaction without any intermediate compounds.

48448

we found the usual shift, for in this length of time the equilibrium between the various intermediates $((Fe^{++}) (Fe^{+++})_3X$, etc.) might well have been established, especially with the ferricyanide-ferrocyanide system also present and probably speeding up the necessary electron transfers. To avoid the latter possibility we did a similar experiment at pH 7.4 with methemoglobin made by Brooks' method. Twenty-four hours' incubation at pH 5.6, $pO_2 = 20$ mm. was, however, necessary to prepare the methemoglobin solution by this method. Unfortunately during this prolonged period a strong reducing agent was found to have developed which rapidly used up the O_2 in the equilibrated samples before analyses could be completed. The nature of this agent was not determined.

Success might be attained with the ferricyanide method if the mixing of the hemoglobin solutions and determination of the oxygen equilibrium could be carried out in a fraction of a second by some rapid flow method.

At very high percentages of methemoglobin only the molecules $(Fe^{++})(Fe^{+++})_3$-X and $(Fe^{+++})_4X$ should be present and according to the law of mass action the dissociation curve should be a rectangular hyperbola, since the only equilibrium to be considered would be that given by the reaction.

$$O_2 + (Fe^{++})(Fe^{+++})_3X = (O_2)(Fe^{++})(Fe^{+++})_3X$$
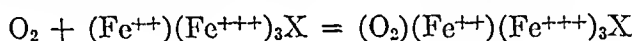
The series of curves in figures 1 and 2 do show a progressive tendency from the S-shape towards the rectangular hyperbola, but we could not work with high enough percentages of methemoglobin (i.e., 95 per cent) to test this idea outright owing to 1, inaccuracies in the O_2Hb estimations with solutions of such low residual O_2 capacity; 2, the possibility that the reversibility of the system O_2Hb—Hb—MetHb—O_2— ferricyanide-ferrocyanide (v. Introduction) might lead to relatively large variations in the concentration of the reduced hemoglobin free to combine with O_2 when the O_2 pressure was varied and the total amount of ferrous hemoglobin was low. The latter was not a significant factor with the percentages of methemoglobin used in figures 1 and 2 but might well become so at percentages > 95.

It seems probable that other hemoglobin compounds such as NO hemoglobin, sulfhemoglobin and the various derivatives of methemoglobin would affect the oxyhemoglobin dissociation curve in the same way as CO-hemoglobin and methemoglobin have been shown to do. We have no evidence on the matter, but would suggest that sulfhemoglobin, and possibly also the so-called "inactive" hemoglobin would be of the most practical interest to study first.

SUMMARY

1. In mixtures of methemoglobin and ordinary hemoglobin the oxygen dissociation curve is shifted to the left as the methemoglobin percentage increases: the shape also becomes progressively less sigmoid and more hyperbolic. The effect is qualitatively the same but quantitatively less than that produced by CO-hemoglobin.

2. The effect has been demonstrated in a, solutions of ox and human hemoglobin in 0.6 M phosphate with methemoglobin made by ferricyanide, aerobic

oxidation or nitrite; *b*, dogs' whole blood during poisoning with nitrite. The size of the effect is the same over the pH range 6.8 to 7.4 and temperature range 25 to 37°C.

3. This effect of methemoglobin is reversible, as is shown by the return of the dissociation curve to normal when the methemoglobin produced in the red cells of the dog by nitrite injection, has had time to be completely reduced by the natural enzymic systems of the red cell.

4. The shifts produced by CO-hemoglobin and methemoglobin are additive.

5. This newly discovered effect means that in methemoglobinemia the tissues are liable to anoxemia, not only from loss of oxygen capacity of the blood, but also from increasing difficulty in the unloading from the blood of such oxygen as is available. Methemoglobinemia should therefore receive more attention in industrial conditions and in drug therapy than hitherto.

6. The mechanism of the effect is believed to be due to the formation of compounds intermediate between reduced hemoglobin (wholly ferrous) and methemoglobin (wholly ferric), the conversion of one or more of the four ferrous atoms in the hemoglobin molecule to ferric leading to an increased affinity of the remaining ferrous atoms for oxygen.

REFERENCES

(1) ADAIR, G. S. J. Biol. Chem., **63:** 529, 1925.
(2) ADAIR, G. S. Proc. Roy. Soc., London A **109:** 299, 1925.
(3) AUSTIN, J. H. AND D. L. DRABKIN. J. Biol. Chem. **112:** 67, 1935.
(4) BARNARD, R. D. J. Biol. Chem. **120:** 177, 1937.
(5) BROOKS, J. Proc. Roy. Soc. B. **118:** 560, 1935.
(6) BROOKS, J. Proc. Roy. Soc. B. **123:** 368, 1937.
(7) CONANT, J. B. AND N. D. SCOTT. J. Biol. Chem. **69:** 575, 1926.
(8) CONANT, J. B. AND L. F. FIESER. J. Biol. Chem. **62:** 595, 1925.
(9) CORYELL, C. D. J. Phys. Chem. **43:** 841, 1939.
(10) CORYELL, C. D., L. PAULING AND R. W. DODSON. J. Physiol. Chem. **43:** 825, 1939.
(11) DILL, D. B., A. GRAYBIEL, A. HURTADO AND A. C. TACQUINI. Ztschr. f. Altersforschung, **2:** 20, 1940.
(12) DOUGLAS, C. G., J. S. HALDANE AND J. B. S. HALDANE. J. Physiol. **44:** 275, 1912.
(13) HALDANE, J. S. Respiration. Yale Univ. Press, New Haven, 1922.
(14) HALDANE, J., M. B. MAKGILL AND MAVROGORDATO. J. Physiol. **21:** 160, 1897.
(15) HARTRIDGE, H. J. Physiol. **54:** 253, 1920.
(16) KEILIN, D. AND E. F. HARTREE. Nature **139:** 548, 1937.
(17) OETTINGEN, W. F. VON. The toxicity and potential dangers of nitrous fumes. Public Health Bull. **272:** U. S. Government Printing Office, Washington, 1941.
(18) PAULING, L. Proc. Nat. Acad. Sci., U. S. **21:** 186, 1935.
(19) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Williams and Wilkins Co., Baltimore, 1931.
(20) STADIE, W. C. AND K. G. MARTIN. J. Clin. Investigation, **2:** 77, 1925.
(21) TAYLOR, J. F. AND A. B. HASTINGS. J. Biol. Chem. **131:** 649, 1939.
(22) WENDEL, W. B. Modern medical therapy in general practice. Williams and Wilkins Co., Baltimore, p. 3015, 1940.
(23) WENDEL, W. B. J. Clin. Investigation, **18:** 179, 1939.
(24) WENDEL, W. B. J. Biol. Chem. **102:** 373, 1933.

CAPILLARY PERMEABILITY AND THE ADRENAL CORTEX STUDIES OF CERVICAL LYMPH IN THE ADRENALECTOMIZED DOG

OLIVER COPE, ADDISON G. BRENIZER, Jr. AND HUGO POLDERMAN[1]

From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital, Boston

Man and experimental animals when suffering from insufficiency of the adrenal cortex are in shock. In 1933 Swingle et al. (1) likened this shock to that seen following trauma and postulated a rôle of the adrenal cortex in surgical shock. Since the publication of Swingle's article the relation of the adrenal cortex to conditions accompanied by low blood pressure and hemoconcentration has been debated.

The low blood pressure and decreased plasma volume found in adrenal cortical insufficiency could be explained by an increased permeability of the capillary wall. Indeed many of the findings in this type of insufficiency in man and experimental animals might be due to an altered permeability of cell membranes in general; it is conceivable that a primary effect of the adrenal cortex might be on the control of permeability. To test this hypothesis it was decided to study permeability where open to direct experimental observation. The permeability of the capillary membrane can be judged by the simultaneous examination of blood plasma and lymph, and this present paper deals with the cervical lymph of the adrenalectomized dog. It has been well established by Drinker and his collaborators (2) that lymph collected from a trunk in the periphery of the body represents essentially extracellular fluid. The walls of the lymphatic channels are so freely permeable to extracellular fluid that only one true cellular membrane, the capillary wall, exists between the blood stream and lymph. Any change in the comparative concentration of the constituents of lymph and blood plasma points, therefore, to an alteration in capillary permeability.

METHODS. To obtain adequate volumes of lymph, dogs were used. All experiments were made without general anesthesia because adrenally insufficient animals have apparently a decreased tolerance to various anesthetic agents, and also to exclude any complicating effect these agents might have upon capillary permeability (3). The dogs were first trained to lie quietly strapped to a table on their back. Under aseptic precautions and with procaine hydrochloride anesthesia (1 per cent solution injected locally into the skin and subcutaneous tissues only) it was possible to cannulize the cervical lymphatic trunk low in the neck. The wound was closed around the cannula for the duration of the experiment. Efforts were made to reproduce the conditions of lymph collection of McCarrell of Drinker's laboratory (4). The flow of lymph was

---

[1] Fellow of the Rockefeller Foundation.

stimulated by flexing manually the head on the neck 15 times per minute; at the end of the minute the lymphatic vessel was emptied by gentle massage into the cannula. The flow of lymph was recorded by weight for each 10 or 20 minute period.

In the animals on which both control and experimental observations were made, the controls were on lymph removed from the trunk of one side. After adrenalectomy the lymph was recovered from the trunk of the other side, the first trunk having been tied off.

The protein of the lymph was measured by the refractometer on approximately each cubic centimeter collected. The refractometer readings were checked in each experiment at at least one point by Kjeldahl nitrogen determinations. The chemical determinations were by standard laboratory procedures. All blood studies were made on arterial blood except for a few of the dye and thiocyanate determinations which were made on blood removed without compression from the external jugular vein. The plasma and extracellular fluid volumes were measured by the method of Gregersen and Stewart (5) during the course of the collection of the lymph. The arterial blood pressure was recorded from a cannula in the femoral artery during the insufficient but not the control period.

EXPERIMENTS. The experiments were divided into two groups; 11 dogs in all were used.

Group 1. Five dogs were adrenalectomized in two stages by the lumbar route. They were then maintained on adrenal cortical extract[2] for four to six days in order to eliminate the complicating effects of operation and anesthesia. Varying amounts of sodium chloride were given in the diet. During the development of insufficiency the animals were fed by hand if they refused to eat in order to minimize the factor of starvation. When the animals were definitely insufficient, but at varying degrees clinically and chemically, both cervical lymphatic trunks were cannulated. Lymph was collected for two or more hours until the rate of flow was established and there was sufficient for the various analyses.

Three unoperated dogs were used as controls.

The data for comparable blood and lymph samples of these eight animals are given in table 1. The lymph figure is an average of several determinations.

Group 2. Three dogs were observed both in the normal and insufficient states, each animal acting as its own control.

After the right adrenal was removed, the control observations on lymph from one cervical trunk and the plasma and extracellular fluid volume determinations were made.[3] When the neck wound was sufficiently healed to exclude infection, the left adrenal was removed. Cortical extract was injected for four to seven days before the animal was allowed to develop insufficiency. A high salt

---

[2] We are indebted to The Upjohn Company for generous gifts of adrenal cortical extract for these experiments.

[3] Although a mild insufficiency may result following the removal of one adrenal (6), it is considered that with the passage of time in a healthy animal the remaining gland undergoes sufficient hypertrophy to give normal function.

TABLE 1

Lymph and blood findings, plasma and extracellular fluid volumes in 5 adrenalectomized and 3 control dogs

	PLASMA (vol. cc.)	EXTRACELLULAR FLUID (vol. cc.)	BLOOD PRESSURE (mm. Hg)	FLOW (mgm./min.)	HEMATOCRIT (% cells)	PROTEIN (gm. %)	PROTEIN (mgm./min.)	AMYLASE (units %)	NPN (mgm. %)	SUGAR (mgm. %)	r. b. (m. Eq./l.)	Na (m. Eq./l.)	K (m. Eq./l.)	Ca (m. Eq./l.)	Cl (m. Eq./l.)	CO_2 (m. Eq./l.)	O_2 CONTENT (vol. %)	O_2 CAPACITY (vol. %)	WEIGHT (kgm.)	INSUFFICIENCY	DATE
Adrenalectomized																					
Dog 1 Blood			55	137	50	7.0	5.7	16.7	71	101	141	141	5.0		115					3+	1.20.41
Lymph						4.1		16.3	31	87	147	142	5.5		115						
Dog 2 Blood			18	213	44	7.8	9.9	16.5	162	41	151	138	8.2	6.4	115				20.7	4+	1.21.41
Lymph						4.6		15.6	171	36	153		7.1		115						
Dog 3 Blood	823	3094	30	59	55	7.0	3.2	18.1	86	75	161	152	6.5	6.4	137	14	23	22		4+	1.24.41
Lymph						4.6		16.5	69	59	163		7.4		137						
Dog 4 Blood	498	3515	75	57	36		2.9	16.1	66	89	150	139	4.9	5.8	118	17	16	17		3+	2.28.41
Lymph						4.8		11.9	73			136	5.0	5.4	118						
Dog 5 Blood			75	129	39	5.4	3.0	18.0	55	78	149	132	4.7		114	21	16	17		3+	3. 5.41
Lymph						2.9		15.2	54	108	145	134	4.8		116						
Control																					
Dog 6 Blood	1104	6147		134	45	6.4	3.6	12.7	23	83	158	144	3.2	4.8	115		′		27.5		7. 9.41
Lymph						2.7		9.5	22	130	160	145		4.6	125						
Dog 7 Blood				59	43	6.3	1.8	11.4	26	82	157	140	3.2	5.2	118	21			18.8		1.22.42
Lymph						3.4		7.7		118	161				118						
Dog 8 Blood			130	89		6.4	2.1	9.6			156	148	3.2	5.2	114				16.0		1.14.42
Lymph						2.6					156	145		4.6	120						

TABLE 2

Lymph and blood findings, plasma and extracellular fluid volumes in 3 dogs before and after adrenalectomy

Sample	DATE	INSUFFICIENCY	WEIGHT (kgm.)	O₂ CAPACITY (vol. %)	O₂ CONTENT (vol. %)	CO₂ (m. Eq./l)	Cl (m. Eq./l)	Ca (m. Eq./l)	K (m. Eq./l)	Na (m. Eq./l)	T. B. (m. Eq./l)	SUGAR (mgm. %)	NPN (mgm. %)	CHOLINE ESTER-ASE (mM/l)	AMYLASE (units 5%)	PROTEIN (mgm./min.)	PROTEIN (gm. %)	HEMATOCRIT (% cells)	FLOW (mgm./min.)	BLOOD PRESSURE (mm. Hg)	EXTRACELLULAR FLUID (vol. cc.)	PLASMA (vol. cc.)
Dog 9 Control Blood	3.19.41			25	24	20	120		4.5	148	156		27	6.6	9.7	2.6	6.1	52	85			1325
Lymph							126			153	160		28	1.9	7.5		3.0				5043	
Adrenect. Blood	4.10.41	3+	26.2	25	24	11	116		7.3	136	146	45	102	10.0	17.1	2.8	7.4	60	61	90		875
Lymph							118		6.6	134	143	94	111	4.3	14.0		4.8				6380	
Dog 10 Control Blood	3.20.41			24	23	23	114	3.3	4.3	148	155		34	5.0	14.8	1.7	3.1	42	59			853
Lymph							120		4.2	146	155		41	1.3	11.9						3521	
Adrenect. Blood	5.23.41	3+	15.7	26	24	21	96	9.6	7.7	126	145	48	124	7.7	18.4	1.2	6.6	59	39	88		450
Lymph							104	6.8		129	142	67	122	1.6	15.3		4.0				3442	
Dog 11 Control Blood	3.10.41			24	22	20	118		3.7	144	151		31		9.9	1.0	6.1	47	50			976
Lymph							126		4.1	150	153		31		6.9		2.2				3190	
Adrenect. Blood	3.29.41	2+		25	23	20	106		4.6	139	146	47	56	9.6	16.8	0.6	7.8	55	17	110		457
Lymph							112			144	155	86		2.3	13.3		4.5				3145	

intake was given by substituting normal saline for drinking water, and food was forced. None of the animals vomited. Finally when the dogs were unsteady but not in severe or terminal insufficiency, the other lymphatic trunk was cannulated. Lymph was collected, the blood pressure followed, and the plasma and extracellular fluid volumes determined.

The results of these experiments are given in table 2; as in table 1, average figures are given when more than one comparable determination was made.

In table 3 is given the flow, the protein concentration and weight of each sample of lymph collected, and the blood pressure throughout the experiment

TABLE 3

Lymph flow, concentration and weight per minute of lymph protein collected from a cervical lymphatic trunk in dog 9 before and after adrenalectomy

CONTROL 3.19.41				ADRENAL INSUFFICIENCY 4.10.41				
	Lymph				Lymph			
		Protein				Protein		
Time	F low	Concen-tration	Weight	Time	Flow	Concen-tration	Weight	Blood pressure
min.	*gm.*	*gm./100/ cc.*	*mgm./min.*	*min.*	*gm.*	*gm./100/ cc.*	*mgm./min.*	*mm. Hg*
20	2.03	3.02	3.1	15	1.35	4.27	3.8	
20	1.44	3.02	2.2	20	1.51	4.35	3.3	90
20	1.67	3.10	2.6	20	1.22	4.63	2.8	90
20	1.54	3.05	2.4	20	1.47	4.71	3.5	90
20	1.76	2.95	2.6	20	1.23	4.89	3.0	
20	1.89	2.97	2.8	20	1.32	4.93	3.3	60
10	0.78	3.10	2.4	20	1.07	5.04	2.7	
Intravenous*				20	0.92	4.89	2.3	
20	1.05	2.94	1.5	15	0.30	5.25	1.1	25
20	1.66	2.68	2.2	Intravenous*				
20	2.39	2.57	3.1	25	0.64	5.17	1.3	45
20	2.48	2.33	2.9	20	1.30	4.50	2.9	
20	2.41	2.40	2.9	20	1.44	4.20	3.0	
20	0.53	2.42	0.6	20	1.05	4.31	2.3	25
20	0.56	2.42	0.7					

* Six hundred cubic centimeters physiologic saline in 20 minutes.

of dog 9. The effect of an intravenous injection of physiologic saline is shown by the figures in this table. Such an injection was given in the three dogs of this group.

RESULTS. *Lymph flow.* The flow of lymph continues in adrenal cortical insufficiency but apparently at a lower rate. The results however are not consistent. The greatest flow observed was in a severely insufficient animal, dog 2. The flow averaged 106 mgm./min. from either cannula in spite of a blood pressure of only 18 mm. Hg. The least flow was 17 mgm./min. in dog 11, an animal in only moderate insufficiency and having a blood pressure of 110 mm. Hg.

Because of the variation encountered in the different animals, both control

and experimental, perhaps the most reliable evidence comes from the animals in group 2 in which each animal acted as its own control. The lymph flow in these three animals was less in the insufficient state. In two there was a 25 per cent reduction, and in the third, dog 11, a 60 per cent reduction.[4] There was no observable correlation between the volume flow and the extracellular fluid volume or blood pressure in these three dogs.

An intravenous injection of 600 cc. of physiologic saline was given the three dogs in group 2 after the determination of the plasma and extracellular fluid volumes. Its effect on the lymph flow was not significantly different in the normal and insufficient states. The entire injection was given within 20 minutes. After a latent period of about 20 minutes the flow of lymph was increased. The results of a typical experiment are shown in table 3.

Protein. The protein content of cervical lymph was increased in the adrenally insufficient dog. The average concentration of all the controls was 2.8 grams/100 cc. and of all the insufficient animals 4.3 grams, an average increase of 1.5 grams, or 54 per cent. The increase above normal was as great in moderate as in severe insufficiency.

In the two animals in severe insufficiency, dogs 2 and 3, the protein concentration of the lymph remained the same throughout the period of collection. Such a stationary concentration of lymph protein was encountered in all of the controls. In the adrenalectomized animals in more moderate degrees of insufficiency there was a steady rise in the protein of the lymph. The difference in protein concentration at the beginning and end of the period of collection amounted to 0.7 to 1.0 gram for the various animals. This rise in protein in the insufficient state is well illustrated in dog 9, table 3, where there was an increase of 1.0 gram in two and a half hours. During this period there was a steady decline in blood pressure. The animal was slipping into shock, presumably owing to the manipulations and withdrawals of blood. Such a rapid decline was seen in the other animals in moderate insufficiency. It is clear that although the protein concentration of the lymph is elevated in moderate insufficiency when the animal is able to walk and even run, there is a further increase as the animal goes into shock.

The weight of protein recovered per minute, since it depended upon the volume of flow, was variable. It was highest in those insufficient animals in group 1 having a big flow, and in this group the insufficient animals averaged higher than the controls. In the second group, two animals put out less protein into the cannula per minute in insufficiency in spite of the increased concentration. Dog 9 (table 3), on the other hand, even with the decreased flow, in insufficiency had a greater output of protein.

---

[4] It should be pointed out not only that the lymph flow varied considerably in the normal animals under what apparently were similar conditions, but also that the pumping of lymph in the normal and insufficient animals may not be comparable. The normal animals resisted the head wagging while the insufficient dogs were apathetic. Such continued muscular resistance, even though mild, may have made the pumping more effective.

The serum protein concentration was increased in the insufficient state as expected. The average of all the controls was 6.3 grams/100 cc. and of all the insufficient animals 7.0 grams, an increase of 0.7 gram.

The averages of both lymph and serum proteins in insufficiency were reduced by the exceptional findings in dog 5. In this animal both proteins were considerably lower than in the other adrenalectomized animals, yet the dog was clinically clearly deficient. During the period of collection, the lymph protein rose from 2.7 grams to 3.6 grams.

The two enzyme systems investigated showed an increased activity in the lymph of the adrenally insufficient animals, a finding consistent with the increase in lymph protein. The amylase activity was increased in the blood serum in all of the adrenalectomized dogs (6). The amylase activity in the lymph in five animals rose proportionately to the rise in the serum; in three (nos. 1, 2 and 3) the activity almost equalled that of the serum, suggesting an increased filtration through the capillary wall of the protein associated with the amylase.

The choline esterase activity (7) showed an increase in both blood serum and lymph in the two animals in which it was measured (nos. 9 and 10). In the first, the increased activity was significantly greater than the rise in serum protein and hematocrit would account for, and in the other it was not. In neither animal was the proportion between the activity in lymph and serum disturbed.

Electrolytes. The concentrations of the total base, sodium and potassium in the lymph varied but little from those in the blood serum in both the normal and insufficient states. Due presumably to the different amounts of salt included in the diet, some of the insufficient animals had normal concentrations of total base and sodium while others were partially depleted. In all deficient animals the potassium was to some degree elevated.

The high calcium level of cervical lymph reported by Heim (8) in dogs under nembutal is confirmed in these experiments for dogs under local anesthesia. The lymph calcium in contrast to spinal fluid calcium is almost as high as that of the serum. The previously reported (9) normal serum calcium level in adrenally insufficient dogs is confirmed with one exception. In dog 10 the serum calcium reached 9.6 m.Eq./l. and the lymph calcium 6.8 m.Eq./l. The increased protein concentrations alone could not account for these findings; they are unexplained.

The chloride concentration of the control lymph is significantly higher than that of the serum, the difference ranging from 6 to 10 m.Eq./l. In the insufficient dog this difference becomes obliterated, presumably due in part to the increase in protein which acts as an acid equivalent. The decrease in CO_2 concentration of the serum (9) is confirmed. The degree of change is irregular and does not fall to 50 per cent of the normal until the late stages of insufficiency.

Non-protein nitrogen. The non-protein nitrogen, elevated in adrenal insufficiency, was sometimes higher, sometimes lower in the lymph than in the serum of the adrenalectomized animals. In the normal dogs the non-protein nitrogen of the lymph was either equal or slightly higher.

Sugar. Differences in sugar concentration between lymph and whole blood were encountered. In the control dogs 6 and 7 the lymph sugar was 36 and 47 mgm. higher than that of the blood.

In insufficiency the sugar levels in general were lower but the lymph sugar level was as often below as above that of the blood. The lymph sugar ranged from 16 mgm. below the blood sugar to 49 mgm. above. The significance of these findings is not clear since plasma sugars before and after fermentation were not done. Heim (10), studying dogs under nembutal, pointed out that if allowances were made for the unequal distribution of sugar between plasma and cells of the blood and for non-fermentable reducing substances in both blood and lymph, there was little or no difference in sugar concentrations between blood plasma and lymph in terms of concentration in water.

Plasma and extracellular fluid volumes. The plasma volume was decreased 44 to 54 per cent in adrenal insufficiency in the three dogs of group 2. Changes in the extracellular fluid volume, in contrast, were not consistent; it was decreased in one, unaltered in another, and increased in the third.

A constant blood pressure graph was determined on two of the animals during and after the injection of the thiocyanate. No significant change in blood pressure occurred in either the normal or insufficient states for the first five minutes from the onset of the injection. This observation was made because in two of the initial observations in the adrenalectomized animals, shock apparently progressed more rapidly after the thiocyanate had been given. It is believed that the shock was due to the blood letting and experimental conditions rather than to any sensitivity of the insufficient dog to thiocyanate.

DISCUSSION. The increased protein content of cervical lymph in the adrenalectomized dog helps to explain the state of shock characteristic of these animals. It has been known for many years that the shock due to lack of adrenal cortical hormone is associated with decreased plasma volume and hemoconcentration. There has been confusion however as to how much these changes were due to water and electrolyte loss from the plasma and how much to total plasma loss. The present experiments offer the first direct evidence that, in addition to any change in water and electrolyte distribution throughout the body, there is an abnormal loss of plasma protein from the blood stream into the extracellular spaces. This leakage of plasma protein is due presumably to an increased capillary permeability.

The disturbance of osmotic equilibrium by such an increase in protein concentration in lymph, and therefore in extracellular fluid, has recently been stressed by McCarrell and Drinker (11). In dogs getting a constant intravenous infusion of histamine they found an increased flow of lymph of high protein content in spite of the lowered blood pressure. The change in capillary permeability induced by the histamine permitted a loss of plasma. They pointed out that the animal was in a precarious situation because the increase in protein of the extracellular fluid reduced the capacity of the plasma to draw fluid back into the blood stream. A situation analogous to that of the dog receiving histamine is found in

adrenal insufficiency. As the increase in capillary permeability continues, the only means for the plasma to replenish itself is by return through the lymphatic channels. As the blood pressure falls and muscular activity decreases, the flow of lymph decreases and the vicious circle between plasma and extracellular fluid is increased. There is little wonder that the adrenalectomized animal should be sensitive to histamine (12).

The protein of the cervical lymph was found to be elevated in moderate as well as in severe insufficiency. Dogs still able to walk briskly had as high a protein concentration as the severely incapacitated animals. The increased protein was therefore not merely an accompaniment of a moribund state. The gradual increase in protein concentration of the lymph during the period of collection in the insufficient animals was probably due to the superimposed, rapidly developing shock induced by the surgical manipulations and the removal of blood.

It is believed reasonable to conclude that the increased protein content and enzyme activity of the lymph points to an increased capillary permeability in adrenal insufficiency. It should be pointed out, however, that the lymph was collected from one region of the body only and that it cannot therefore be concluded that a generalized increase in capillary permeability exists. Lymph from other organs and regions will first have to be examined.

The significance of the extracellular fluid volume determinations is open to question. Ferrebce et al. (13), using the thiocyanate method in patients with Addison's disease, found a decrease in extracellular fluid as well as in plasma volume. Thorn (14), also studying patients, obtained such variable results that he considered the method unreliable in adrenal insufficiency. He suggested that cells, in the absence of the cortical hormone, might become permeable to thiocyanate. Such an alteration of cell permeability could account for our own results. On the other hand if the intakes of salt and fluid are maintained during the period when the animal is developing the signs of insufficiency, it could well be that the extracellular fluid volume would actually be increased above normal. If the decreased concentration of thiocyanate in the plasma were to be explained by entrance of thiocyanate into cells, it should occur in all animals in the insufficient state and the differences encountered would still have to be explained on the basis of different fluid volumes, extra- and intra-cellular.

Too much emphasis should not be attached to the observed rates of lymph flow because of the variations encountered. The important thing is that in those animals having a high rate of flow in the insufficient state, the protein concentration was maintained, indicating that the high protein content was not due to mere stagnation of extracellular fluid but to capillary filtration.

SUMMARY AND CONCLUSIONS

Lymph was collected from the cervical trunk of normal and adrenalectomized dogs under local anesthesia. The protein content of the lymph of the normal animals averaged 2.8 grams/100 cc., and of the adrenalectomized 4.3 grams. The increase above normal was as great in the dogs in moderate as in severe in-

sufficiency and was not merely an accompaniment of a moribund state. The finding offers direct evidence of an increase in capillary permeability in one region of the dog in adrenal insufficiency.

The significance of this protein increase in lymph to the osmotic equilibrium between blood plasma and extracellular fluid is discussed.

The flow of lymph was in general, but not consistently, reduced in insufficiency. The extracellular fluid volume, measured by means of thiocyanate, was increased as well as decreased.

REFERENCES

(1) SWINGLE, W. W., J. J. PFIFFNER, H. M. VARS, P. A. BOTT AND W. M. PARKINS. Science 77: 58, 1933.
(2) DRINKER, C. K. AND J. M. YOFFEY. Lymphatics, lymph and lymphoid tissue. Harvard Univ. Press, Cambridge, Mass., 1941.
(3) BEECHER, H. K. AND H. POLDERMAN. Unpublished data.
(4) McCARRELL, J. D. This Journal 126: 20, 1939.
(5) GREGERSEN, M. I. AND J. D. STEWART. This Journal 125: 142, 1939.
(6) COPE, O., I. KAPNICK, A. LAMBERT, T. D. PRATT AND M. G. VERLOT. Endocrinology 25: 236, 1939.
(7) COPE, O., O. KRAYER AND F. PLACHTE. To be published.
(8) HEIM, J. W. This Journal 103: 553, 1933.
(9) HARROP, G. A., L. J. SOFFER, R. ELLSWORTH AND J. H. TRESCHER. J. Exper. Med. 58: 17, 1933.
(10) HEIM, J. W., R. S. THOMSON AND F. C. BARTTER. This Journal 113: 548, 1935.
(11) McCARRELL, J. D. AND C. K. DRINKER. This Journal 133: 64, 1941.
(12) DALE, H. H. Beitr. J. Exper. Path. 1: 103, 1920.
(13) FERREBEE, J. W., C. RAGAN, D. W. ATCHLEY AND R. F. LOEB. J. A. M. A. 113: 1725 1939.
(14) THORN, G. W. Personal communication.

THE PROPHYLACTIC ACTION OF DESOXYCORTICOSTERONE IN SHOCK DUE TO MASSIVE VENOUS THROMBOSIS[1]

L. N. KATZ, S. T. KILLIAN, R. ASHER and S. PERLOW

From the Cardiovascular Department, Michael Reese Hospital, Chicago

Since the synthesis of the adrenal cortical steroid, desoxycorticosterone acetate (DCA), by Reichstein in 1938 (1), many workers have investigated its physiological and pharmacological properties. DCA has been suggested in the treatment of the shock syndrome but at present considerable confusion exists concerning its value in this condition.

Swingle and his associates (2) have shown that DCA protects adrenalectomized dogs from circulatory collapse and shock produced by muscle trauma, injections of epinephrine, and the fluid and salt loss attendant upon the intraperitoneal injections of isotonic glucose solutions, but does not prevent shock following intestinal manipulation. Perla and his co-workers (3) demonstrated that DCA combined with saline solution increased the resistance of mice to histamine shock. The beneficial effects of DCA in shock following severe burns has been shown experimentally by Wilson and Stewart (4).

On the other hand, Selye and his co-workers (5) found no benefit to accrue from DCA in preventing formaldehyde or intestinal trauma shock in rats and they point out that large doses of DCA may be dangerous because of deleterious effects on the adrenals. Weil et al. (6) were unable to show that DCA caused any significant reduction in the mortality of rabbits subjected to intestinal manipulation.

Clinically, Perla et al. (3) claimed beneficial effects from DCA therapy in a small series of patients subject to surgery. Wilson and Stewart (4) found it of value in burns. However, Keating, Power and Rynearson (7) found no beneficial effects of DCA in women undergoing radical mastectomy for breast carcinoma.

Obviously results from studies done with adrenalectomized animals need not apply to those done with normal animals. It is also apparent that various forms of shock may respond in different degrees to DCA therapy. Furthermore, the difficulty of evaluating clinical experience with prophylactic procedures to prevent shock can easily lead to the discrepancies in the literature.

Recently we have described a method in which occlusion of the venous return from one limb of the dog led to a massive edema of that limb and a state of shock followed by death within $3\frac{1}{2}$ to 21 hours (8). Since this procedure appeared to give consistent results we felt that the action of DCA could be explored in such animals. We therefore attempted to evaluate the prophylactic and therapeutic action of DCA in a series of such dogs.

METHOD. In this study we have used three series of dogs: a, *controls*, a group of fifteen dogs; b, *primed* dogs, a series of eleven dogs which received, on the average, 25 mgm. of DCA[2] in divided doses during the 24 hours preceding the operation, and an average of 35 mgm. of DCA during the following 24 hours; c, *unprimed* dogs. In this "unprimed" series, three dogs received an average of 40 mgm. of DCA starting two hours after the operation and continued over a period of twelve hours, and six other dogs received 10 mgm. of DCA 10 minutes to $2\frac{1}{2}$ hours pre-operatively and an average of 36 mgm. during the following twelve hours.

TABLE 1

Controls

DOG	WEIGHT	WEIGHT INCREASE OF EDEMATOUS LEG OVER CONTRALATERAL ONE		DURATION OF LIFE AFTER OPERATION
no.	*kgm.*	*grams*	*% of body weight*	*hours*
1	15.4	750	4.8	$9\frac{1}{2}$
2	13.2	702	5.3	$6\frac{1}{2}$
3	12.7	503	4.0	$13\frac{1}{2}$
4	9.5	570	6.0	5
5	13.6	737	5.4	6
6	10.9	668	6.1	$4\frac{1}{2}$
7	10.5	458	4.3	10
8	11.8	576	4.9	$5\frac{1}{4}$
9*	11.0	250	2.3	Survived
10	12.0	773	6.4	7
11	15.4	363	3.5	$3\frac{1}{2}$
12	9.9	486	4.8	8
13	11.3			Survived
14	11.8	680	5.7	$10\frac{1}{2}$
15	11.0	690	6.9	12–21†

* Sacrificed on 3rd day after operation; edema in leg has diminished.
† Animal died between 11:30 p.m. and 7:30 a.m.
Total number, 15; died in shock, 13; mortality, 87%.

The observations in these experiments were the same as those outlined in our previous report: hematocrit, blood pressure, heart rate, size of leg, etc. (8).

RESULTS. The results of the three series are shown in tables 1, 2, and 3 respectively. Typical charts of the latter two series are seen in figures 1 and 2 for comparison with the control series (8).

The observations in the present control series of 15 (summarized in table 1) coincided with those previously reported, with the exception that two dogs survived. In those that succumbed, the post-operative sequence was similar to those previously reported.

In the animals primed with DCA, the post-operative symptoms were similar but milder than those of the control series. Although the occluded limbs be-

came cold and severely edematous, the dogs appeared normal, drank water and appeared to be in a good condition. Two of these animals (nos. 1 and 4, table 2) receiving a smaller quantity of DCA pre-operatively (0.9 and 1.6 mgm./ᴋ) died in shock in 4½ and 29 hours respectively. One dog with distemper (no. 11), died in 12 hours with pulmonary edema. Eight out of 11 animals survived. The blood pressure in the dogs that survived remained unchanged (fig. 1) or was slightly reduced. Hemoconcentration, as measured by the hematocrit, was
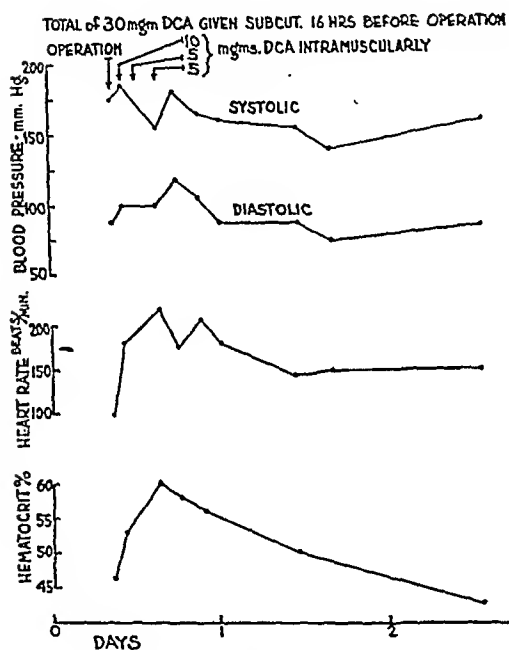
Fig. 1

Fig. 2

Fig. 1. A typical experiment in which desoxycorticosterone acetate was given before as well as after the shock producing operation—"primed animal." Note that time scale is in days. Discussed in text.
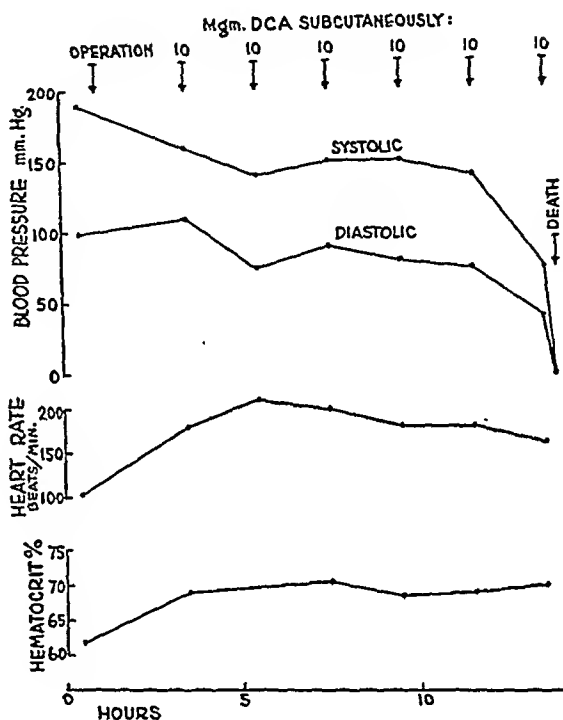
Fig. 2. A typical experiment in which desoxycorticosterone acetate was given only after the shock producing operation—"unprimed animal." Note that time scale is in hours. Discussed in text.

evident during the first several hours post-operatively, to an extent almost as great as in the control series, but returned toward normal levels the following day, followed in some by a long period of hemodilution. This reduction in red blood cell concentration was coincident with a gradual return of the edematous leg to normal.

The results in the unprimed series were identical with those of the control series (table 3). Seven animals died in shock, one died of pneumonia and only one dog survived. The postoperative picture, as shown in part in figure 2, was almost identical with the control series.

TABLE 2

Dogs primed with DCA

DOG	WEIGHT	TOTAL MGM. DCA INJECTED		DURATION OF LIFE	WEIGHT INCREASE OF EDEMATOUS LEG
		Pre-operative	Post-operative		
no.	kgm.			hours	% of body weight
1*	15.5	15, IM	10, IM	29	4.4
2	13.0	20, IM	30, IM	Survived	
3	11.0	15, IM	5, IM	Survived	
4*	9.0	15, IM	20, IM	4½	3.4
5	10.5	30, IM	130, IM	Survived	
6	12.2	30, Sub Cut.	50, IM	Survived	
7	13.6	30, Sub Cut.	20, IM	Survived	
8	12.7	30, Sub Cut.	40, IM	Survived	
9	12.2	20, Sub Cut.	40, IM	Survived	
10	12.2	35, Sub Cut.	15, IM	Survived	
11†	12.7	40, Sub Cut.	40, Sub Cut.	12	7.3

* Died in shock.
† Died with pulmonary edema.
Total number, 11; survived, 8; mortality, 27%.
IM = intramuscularly; Sub Cut. = subcutaneously.

TABLE 3

Dogs receiving DCA post-operatively

DOG	WEIGHT	TOTAL MGM. DCA INJECTED		DURATION OF LIFE	WEIGHT INCREASE OF EDEMATOUS LEG OVER CONTRALATERAL ONE	
		Pre-operative*	Post-operative			
no.	kgm.			hours	grams	% of body weight
1	9.5	10, IM	40, IM	4½	190	2.0
2	11.3	10, Sub Cut.	30, Sub Cut.	Survived		
3†	12.2	10, Sub Cut.	40, IM	15	694	5.7
4	11.0	10, Sub Cut.	50, IM	15	690	6.3
5	8.2	10, Sub Cut.	30, IM	7	396	4.8
6	13.1	10, Sub Cut.	30, Sub Cut.	11	1175	8.9
7	13.6	None	30, Sub Cut.	20	676	4.9
8	10.4	None	40, Sub Cut.	12	665	6.4
9	10.9	None	60, Sub Cut.	12	642	5.8

* Administered 10 min. to 2¼ hrs. pre-operatively.
† Died with pneumonia.
Total number, 9; died in shock, 7; mortality, 78%.
IM = intramuscularly; Sub Cut. = subcutaneously.

DISCUSSION. Our results indicate that when DCA is given in adequate quantities (1.3 to 3.0 mgm./K) for the 24 hours preceding the shock-initiating operation, it is an effective agent in preventing the type of shock produced in our experiments. If the administration of DCA is delayed the prophylactic effect is reduced and may be inadequate to prevent shock. It must be borne in mind that

even though DCA is a valuable prophylactic agent in this form of shock, it may not be efficacious in other forms of shock.

In the present experiments DCA might operate in one or more ways: 1. It might maintain the normal permeability of the capillaries or alter the forces controlling the exchange of fluid between blood and tissues so that the rate of escape of fluid from the blood might be lessened.   2. It might alter the water balance *a*, by decreasing the renal excretion of fluid; *b*, by enhancing thirst; *c*, by lessening the tendency to vomit; or *d*, by affecting the salt-water balance between the blood and tissues.   In one or another of these ways a more positive water balance would be favored with consequent less strain on the extracellular and vital in-
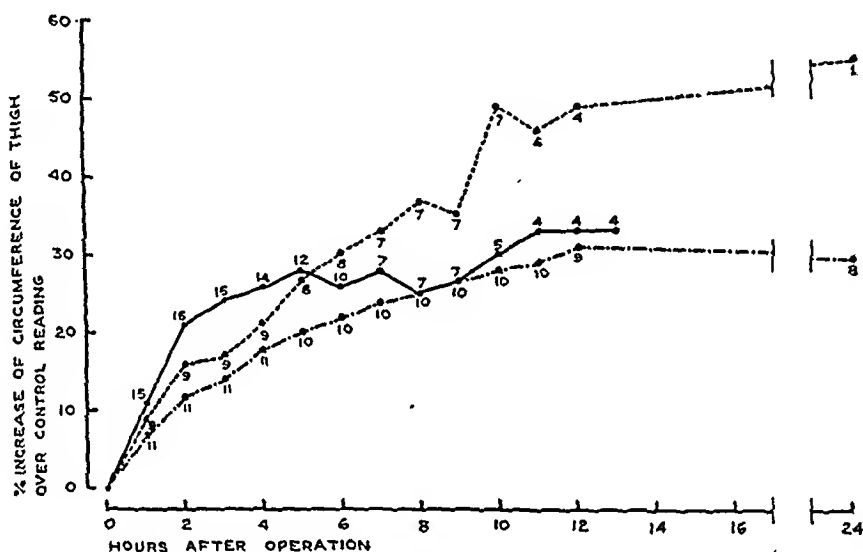
Fig. 3. A graph summarizing the changes in mid-thigh circumference observed in the control (solid line), the unprimed (dash line) and the primed (dot-dash line) series following the shock-producing operation.   The change in circumference in each series is the average increase over the value existing before the operation in the dogs which survived at each time interval.   The number of animals surviving at each time interval in each series is shown by the figures on each curve.   Discussed in text.

tracellular water compartments.   3. It might operate directly on the cardiovascular system, and so aid in maintaining peripheral vascular tone, or delay or prevent the decrease in cardiac activity which contributes to making shock irreversible.   4. It might counteract a humoral toxic agent released from the edematous limb.

In the course of our studies circumferential measurements made of the edematous leg at mid-thigh and mid-calf showed that the size of the leg tended to increase in the same proportion at these two levels.   In figure 3 is plotted the average values for mid-thigh, expressed as per cent increase over the circumference before operation, together with the number of animals on which these measurements were made at each time interval.   The decrease in these figures

indicates the number of deaths in the preceding hour. Certain trends are apparent, even though the measurements were necessarily crude.·

If the curve depicting the average increase in circumference of the edematous leg in the control series is followed, it will be seen that it flattens out as soon as the mortalities occur, indicating that the animals with the greater rate of leg enlargement died sooner. This confirms our previous observation (8) that the time of death of the animals was roughly in inverse proportion to the rate of fluid accumulation in the leg (as can be seen, table 1, by the value of

$$\frac{\text{per cent increase in edematous leg weight at necropsy}}{\text{body weight of dog}}$$

divided by the time of post-operative survival). In other words, with animals with the greater rate of accumulation of fluid succumbing, the curve must flatten out. The slight flattening out of the curve before mortalities occur could be explained by the increased oncotic pressure associated with hemoconcentration and the increase in tissue pressure as edema developed, both of which tend to lessen the escape of fluid into the leg.

More significant is the fact that during the first five hours, the average thigh circumference of the primed DCA series was less than that of the unprimed DCA series and the latter less than the control series. While the differences are not great, the trend unmistakably indicates that DCA tends to diminish the loss of fluid into the occluded limb. Since the results from this laboratory indicate that DCA is without effect on capillary permeability (9, 10), the mode of action must be on either the hydrostatic or oncotic pressure in the blood. The fact that the blood pressure, and presumably the venous and capillary pressures, drops more in the control and unprimed series than in the primed series in this time interval, would rule out the hydrostatic factor, which in fact would work in the opposite direction. So, by exclusion, it would imply an action on the oncotic pressure of the blood favoring retention of fluid in the blood. This action of DCA is further substantiated by comparing the rate of increase in leg size in the primed and unprimed series after the fifth hour.

Thus, this analysis shows that one of the main differences between the three series appears to depend on the rate of accumulation of fluid in the occluded limb. The delay in death of the unprimed dogs is probably due to the delayed accumulation of fluid in the leg. In fact, the still slower accumulation of the fluid in the primed dogs may explain, in part, their survival. The manner by which the oncotic pressure changes are induced is not revealed in these experiments.

It will be noted that death occurred in the control series after an amount of fluid equivalent to about 4 per cent of the total body weight had accumulated in the edematous leg (table 1). In the primed series the rate of fluid accumulation was reduced but despite the fact that the leg size increased ultimately almost as much as in the control series, shock and its sequelae were prevented by DCA. This indicates that the action of DCA in preventing shock is not solely on the loss of fluid. This is evidenced further by the fact shown in figure 3 that the rate

of hemoconcentration could not be related to the rate of increase in leg size or to the development of shock. Furthermore, no correlation could be established in the rate of hemoconcentration in the three series. These findings suggest that hemoconcentration is not an infallible, quantitative guide of the development or occurrence of shock, even when plasma loss appears to be the initiating mechanism as in our experiments. For example, in dog 15, table 1, no hemoconcentration was noted in the first 12 hours of post-operative observation, and yet toward the end of this period the blood pressure definitely began to fall.

The loss of fluid from the blood as shown by the hematocrit is directly correlated with the rate of fluid accumulation in the leg during the first few hours. Furthermore, the homeostatic mechanism which tends to restore the ratio of red blood cells to plasma volume per cubic centimeter of blood takes several hours to manifest itself. Once it is set in motion this mechanism continues and "overshoots" leading to a hemodilution. This lag in the action of the mechanisms compensating for the loss of fluid from the blood makes it possible for rapid fluid loss, as occurred in our experiments, to be incompletely compensated and thus permit the development of an irreversible state before the adjusting mechanisms are in full operation.

It is interesting in this connection that upon analysis the edematous leg of the unprimed DCA dogs reached a higher circumferential measurement after the fifth hour than did the control dogs, thus suggesting that they could survive a greater loss of fluid because DCA was given. Our results indicate that the irreversibility of shock is not dependent solely on the loss of blood or plasma volume but also upon the secondary changes which this sets into operation.

The mechanisms by which DCA operates is not revealed by our experiments, but it is clear that it lessens the intensity of some of the deleterious processes which induce the vicious cycle leading to irreversibility of the shock condition. Its value as a prophylactic agent, when used early enough and in adequate dosage, in those forms in which loss of plasma is the initiating factor seems established. It probably may also be of value prophylactically in other forms of shock, but its precise utility must await further analysis of its actual mode of action.

SUMMARY

1. Experimental massive venous occlusion of a leg leads to a fall in blood pressure, a rise in hematocrit and an increase in the size of the leg amounting to 2.3 to 6.9 per cent of the body weight. This results in death in $3\frac{1}{2}$ to from 12 to 21 hours. Only an occasional animal survives (2 out of 15 in our series).

2. The administration of desoxycorticosterone acetate (DCA) over a period of 24 hours previous to, and during the first 24 hours after the onset of the venous occlusion prevents the development of the state of shock and the animals survive (8 out of 11 in our series) despite a loss of fluid comparable to that in the control series.

3. When the DCA is *not* given sufficiently early before the onset of venous oc-

clusion, the picture of shock and the mortality are similar to those of the untreated animals (death occurred in 8 out of 9). However, the average time of death is delayed somewhat and the loss of fluid is greater.

4. Evidence is given to show that DCA decreases the rate of fluid loss due presumably to some action on the oncotic pressure of the blood.

5. The action of DCA in preventing shock and the development of an irreversible state is due to some other mechanism in addition to its action on fluid loss from the blood. The nature of this action was not revealed by these studies.

REFERENCES

(1) REICHSTEIN, T. AND J. V. EUW. Helvet. Chim. Acta 21: 1197, 1938.
(2) SWINGLE, W. W., H. W. HAYS, J. W. REMINGTON, W. D. COLLINGS AND W. M. PARKINS. This Journal 132: 249, 1941.
(3) PERLA, D., D. G. FREIMAN, M. SANDBERG AND S. S. GREENBERG. Proc. Soc. Exper. Biol. and Med. 43: 397, 1940.
(4) WILSON, W. C. AND C. P. STEWART. Trans. Med Chir. Soc. Edinburgh, Edinburgh M. J. 46: 153, 1939.
(5) SELYE, H., C. DOSNE, L. BASSETT AND J. WHITTAKER. Canadian M. A. J. 43: 1, 1940.
(6) WEIL, P. G., B. ROSE AND J. S. L. BROWNE. Canadian M. A. J. 43: 8, 1940.
(7) KEATING, F. R., JR., M. H. POWER AND E. H. RYNEARSON. Proc. Staff Meet. Mayo Clinic 17: 59, 1942.
(8) PERLOW, S., S. T. KILLIAN, L. N. KATZ AND R. ASHER. This Journal 134: 755, 1941.
(9) FREED, S. C. AND E. LINDNER. This Journal 134: 258, 1941.
(10) FREED, S. C. AND I. H. SHLESER. Unpublished.

EFFECT OF VAGOTOMY AND OF SYMPATHECTOMY ON THE SENSITIVITY OF INTESTINAL SMOOTH MUSCLE TO ADRENALIN[1]

W. B. YOUMANS, A. I. KARSTENS AND K. W. AUMANN[2]

From the Department of Physiology, University of Oregon Medical School, Portland

Evidence that sectioning of visceral efferent nerves results in hypersensitivity of the denervated effectors to the action of adrenalin has been derived mainly from experiments involving excitatory adrenergic nerves. Less evidence is available with regard to cholinergic and inhibitory adrenergic neuro-effector systems. Cannon (1) has recently reviewed the literature and has formulated a "law of denervation." Studies testing the applicability of the generalization to inhibitory adrenergic nerves are few and contradictory. Elliott (2) (3) and Langley and Magnus (4), who were interested in the site of action of adrenalin rather than the quantitative aspects of the problem, reported normal reactions of effectors to adrenalin after sectioning of their inhibitory adrenergic nerve supply. More recent workers who have designed their experiments for the purpose of determining if altered sensitivity to adrenalin occurs following destruction of inhibitory adrenergic nerves have, in most cases, reported sensitization. The effectors utilized have been perfused guinea pig bronchioles (5), rabbit intestine (6) (7) (8) (9) (10) isolated or *in situ* in acute experiments, the non-pregnant feline uterus in acute experiments (9), and the unanesthetized dog intestine *in situ* (11). One investigator (12), utilizing the dog intestine in acute experiments, reported a reversal of the action of adrenalin following denervation. In the studies by Drake el al. (10) on rabbit intestine precautions were taken to determine separately the effects of sympathetic and parasympathetic denervation. In the study utilizing unanesthetized dogs (11) it was demonstrated that extrinsically denervated Thiry fistulae of the jejunum were hypersensitive to the inhibitory action of adrenalin. The present experiments have been performed to determine the effects of various operations upon autonomic pathways to the intestine on the sensitivity of intestinal smooth muscle to the inhibitory action of adrenalin.

METHODS. Dogs were prepared each having two Thiry fistulae made from adjacent segments of the upper jejunum. Following recovery from the operation the sensitivity of these loops to adrenalin was determined by injecting adrenalin solutions intravenously from a motor-driven syringe. An adrenalin injection rate was determined in each case that would produce submaximal inhibition of intestinal motility as recorded by balloon-mercury-manometer systems. Effects of injections at one-half and twice this rate were also recorded.

Precautions used in preparing and injecting the solutions were the same as those described in the previous quantitative study (11). The animals were then subjected to either vagotomy or sympathetic decentralization of the preaortic ganglia. Sensitivity to adrenalin was re-determined, and then one of the two
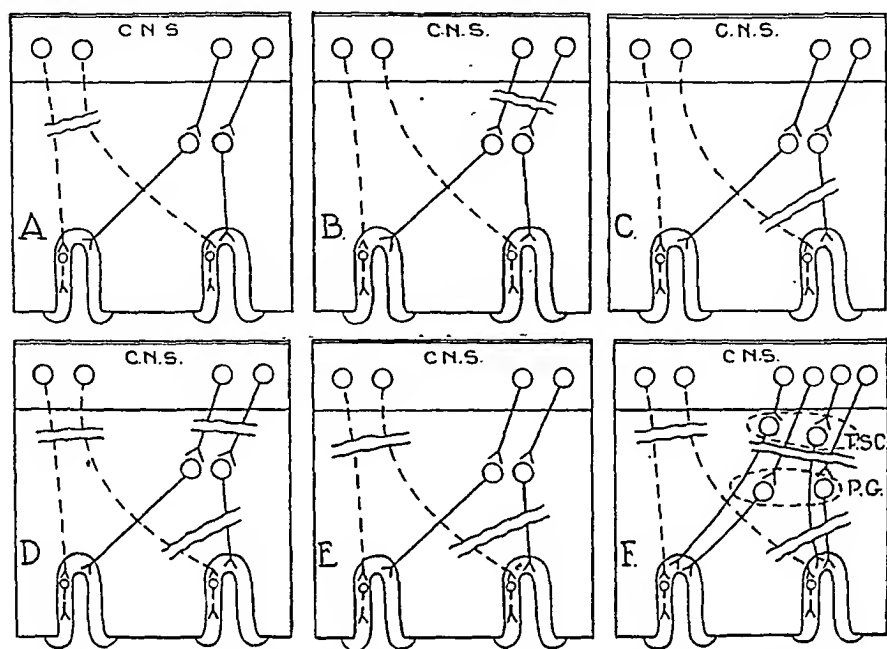
Fig. 1. A–F. Diagrammatic representation of the innervation of intestinal segments remaining after various operations. Possible variations from this schema are discussed in the text. Vagal pathways are indicated by broken lines; sympathetic pathways are represented by continuous lines, and the double waved lines indicate the location of the interruption of pathways with reference to the first and second neurones in the visceral efferent pathways.

A. Vagotomy,—preganglionic interruption of parasympathetic innervation of both loops.

B. Splanchnicotomy and lumbar ganglionectomy,—destroys sympathetic connections with the pre-aortic ganglia but leaves intact the nerve fibers passing to the intestine from cell bodies in the pre-aortic ganglia.

C. Mesenteric denervation,—destroys preganglionic parasympathetic and postganglionic sympathetic innervation of the segment.

D. Combination of A, B and C,—results in destruction of all connections between the pre-aortic ganglia and the central nervous system (C.N.S.), and one of the segments has its connections with the pre-aortic ganglia destroyed.

E. Combination of A and C,—one segment has its sympathetic connections with the central nervous system intact, while the other has its sympathetic connections with the pre-aortic ganglia destroyed.

F. Alternative diagram of D.

loops in each animal was denervated by sectioning the nerves in the mesentery. The vagotomies were done by sectioning the nerves along the lower esophagus. Sympathetic decentralization of the pre-aortic ganglia was accomplished by sectioning the splanchnic nerves and removing the lumbar sympathetic chains. The completion of sympathetic denervation of the intestine was indicated by

elimination of intestino-intestinal reflexes and absence of a pain response to intestinal distention. In some of the animals sensitivity to adrenalin was determined after all three of the operations had been completed. The purpose when studying the effect of any one set of nerves was to have the two loops differ from each other only with regard to that set of nerves. This was not possible in every case, and it was necessary to record the sensitivity of the same loop for a period of time before and after denervation. A diagrammatic representation of the innervation of the Thiry fistulae remaining after completion of the various operations or combinations of operations is contained in figure 1, A–F. However, anatomical information concerning the intestinal innervation is inadequate; therefore, no interpretations are based on the accuracy of the diagrams.

RESULTS. I. *Effect of vagotomy on the sensitivity of intestinal smooth muscle to adrenalin.* A comparison of responses to intravenous injection of adrenalin before and after vagotomy was made in each of five dogs. In two of these animals a mild sensitization appeared to have occurred. However, in the other three cases there was no definite evidence of alteration of sensitivity. Results from the five animals indicate that vagotomy either has no effect on the sensitivity of intestinal smooth muscle to adrenalin, or it produces a mild sensitization.

These results are essentially in agreement with those of Drake, Modern, Renshaw and Thienes (10) who concluded that no alteration occurred in the response of the isolated rabbit intestine to adrenalin following vagotomy. However, their conclusion was based on comparison of responses of segments of intestine from vagotomized animals with segments from other animals with the vagi intact. In view of the wide variation in sensitivity of loops of intestine from different rabbits, it seems that sensitization would need to be considerable in order to be detected by this method.

II. *Effect of sympathetic decentralization of the pre-aortic ganglia on sensitivity of intestinal smooth muscle to adrenalin.* Sensitivity of intestinal loops to adrenalin was studied in four dogs before and after sympathetic decentralization of the pre-aortic ganglia and plexuses by means of bilateral splanchnicotomy and removal of the lumbar sympathetic chains. In three of these animals mild sensitization occurred. The maximal increase in sensitivity was less than twofold. In the other animal there was no definite evidence of sensitization.

Modern and Thienes (8) reported that sectioning of the splanchnic nerves did not alter the response of excised segments of rabbit intestine to adrenalin. However, Drake, Modern, Renshaw and Thienes (10) reported that splanchnicotomy results in a two-fold increase in the sensitivity of isolated intestinal strips to adrenalin. The variable results reflect the difficulty of detecting sensitization by the isolated strip technique when the sensitization is slight.

III. *Effect of complete decentralization of the pre-aortic ganglia on sensitivity of intestinal smooth muscle to adrenalin.* Combination of vagotomy, splanchnicotomy, and lumbar ganglionectomy eliminates all nervous connections between the central nervous system and the pre-aortic ganglia. The intestinal loops of three dogs having had this combination of operations were only mildly sensitized to adrenalin. The maximal sensitization was not greater than twofold.

IV. *Sensitization of the intestine to adrenalin by sectioning nervous connections*

between the pre-aortic ganglia and the intestine. The previous experiments (11) demonstrated that mesenteric denervation of a loop of intestine produces a three-to seven-fold increase in sensitivity to adrenalin. According to the usual interpretation of intestinal innervation mesenteric denervation would result in preganglionic vagal denervation and postganglionic sympathetic denervation. In the present study the effect of the mesenteric denervation has been determined in vagotomized animals some of which also had the pre-aortic ganglia sympathetically decentralized.
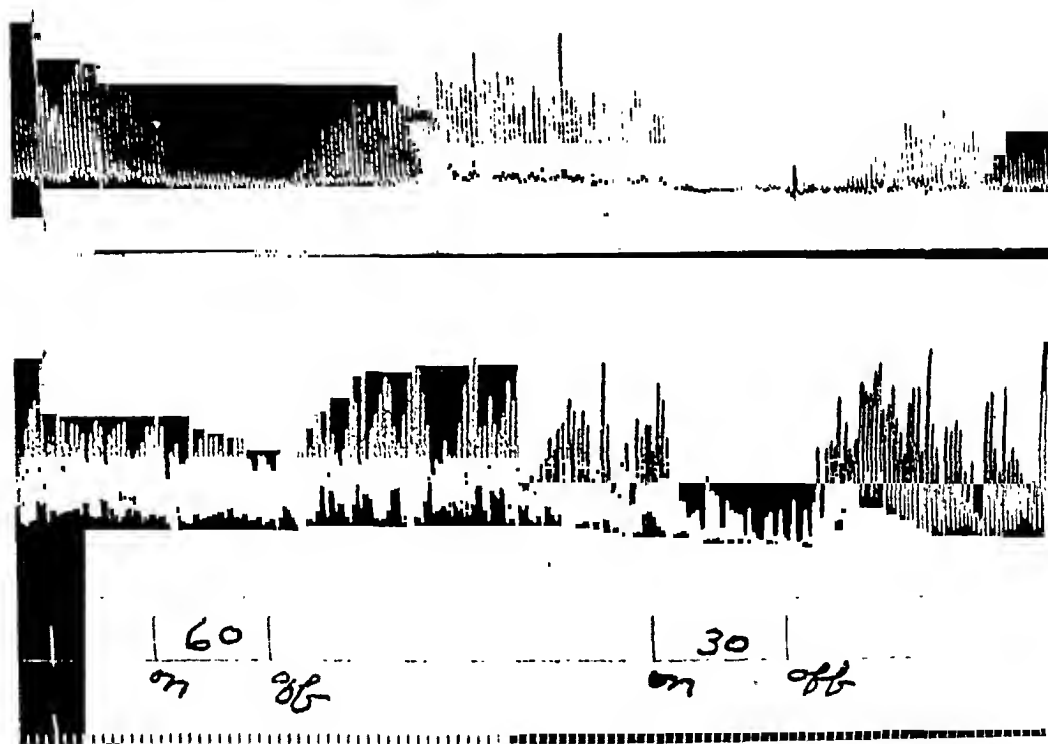
Fig. 2. Effect of adrenalin, 1–250,000, injected at rates of 1 and 2 cc. per minute, respectively, on the motility of a mesenterically denervated intestinal segment (upper record) compared with a simultaneous record (below) from a segment having its connections with the pre-aortic ganglia intact. The pre-aortic ganglia have been decentralized by vagotomy, splanchnicotomy, and lumbar ganglionectomy. A diagrammatic representation of the nervous pathways remaining after the denervations is shown in figure 1, D.

a. *Pre-aortic ganglia completely decentralized.* Three animals were prepared as illustrated diagrammatically in figure 1, D. Simultaneous records were taken from the two loops in each of these animals during the injection of adrenalin. The two loops in a given animal differ from each other in that one retains its innervation from cell bodies in the decentralized pre-aortic ganglia while the other does not. In each of the three dogs the loop having no nervous connections with the decentralized ganglia was three to six times more sensitive to adrenalin than the loop having its connections with the ganglia intact. A typical record is shown in figure 2.

b. *Pre-aortic ganglia sympathetically or vagally decentralized.*   Two animals had the pre-aortic ganglia sympathetically decentralized and, in addition, one of the loops in each animal was mesenterically denervated, but the vagi were intact. The results in these animals were indistinguishable from those described in the preceding paragraph.   The mesenterically denervated loops were three to seven times more sensitive to adrenalin than the other loops.

Some of the animals were vagotomized and, in addition, had one of the loops mesenterically denervated.   The pathways remaining intact after these opera-
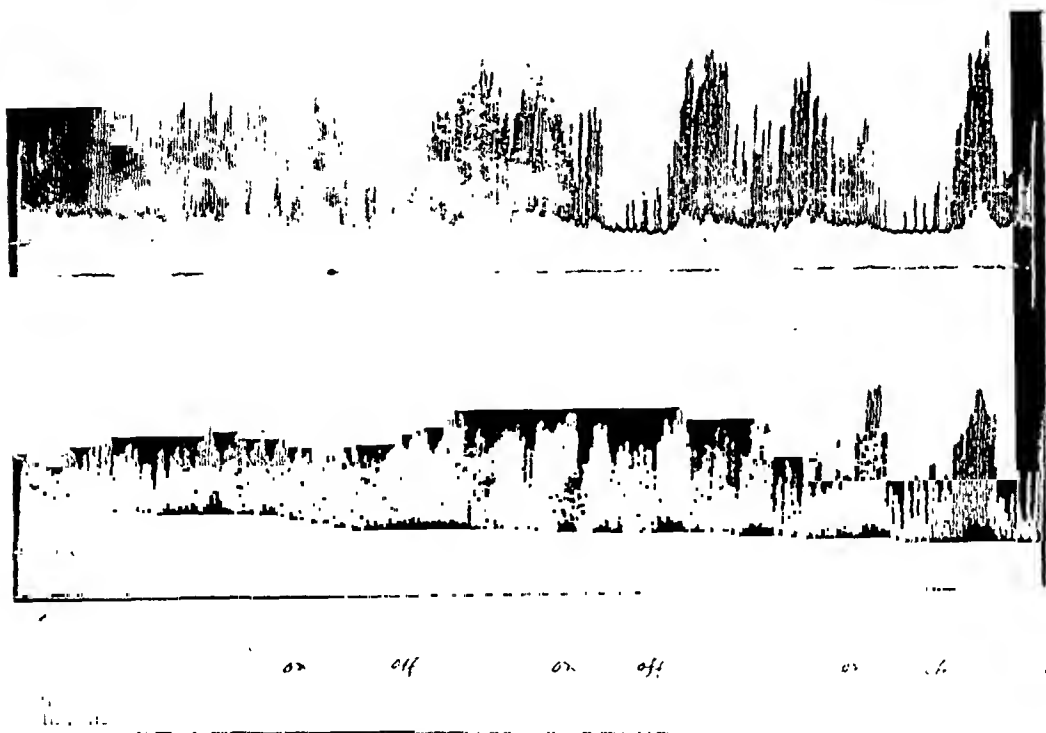
Fig. 3. Effect of adrenalin, 1–1,000,000, injected at rates of 1, 2 and 4 cc. per minute, respectively, on the motility of a mesenterically denervated intestinal segment (upper record) compared with a simultaneous record (below) from a segment having only its vagal innervation destroyed. The innervation remaining to each of the loops is represented diagrammatically in figure 1, E.

tions are shown diagrammatically in figure 1, E.   In these animals the denervated loop was in each case three to six times more sensitive to adrenalin than the sympathetically innervated loop.   Subsequent sympathetic decentralization of the pre-aortic ganglia, resulting in the preparation described in a., only mildly reduced the difference in the sensitivity of the two loops.   A record obtained from a vagotomized animal having one loop mesenterically denervated is shown in figure 3.

V. *Rate of development and duration of hypersensitivity after mesenteric denervation.*   In some animals the denervated loop shows sufficiently regular motility within two days after the mesenteric denervation so that sensitivity to adrenalin

may be tested. Tests repeated every two days for the period of ten days after the operation indicated that almost maximal hypersensitivity to adrenalin was attained by the time of the first test (two days after the operation). The hypersensitivity persists well beyond the time when regeneration of nerves is to be expected. The denervated loop of one of the dogs used in the former study (11) was more sensitive than the innervated loop three years after the time when the nerves were sectioned in the mesentery.

DISCUSSION. If the disposition of cell bodies represented diagrammatically in figure 1 is correct the increased sensitivity of one of the intestinal segments following mesenteric denervation in an animal with pre-aortic ganglia decentralized is attributable entirely to sectioning of postganglionic sympathetic fibers. However, if any vagal fibers synapse in the coeliac ganglion the interpretation of the results would be more complex. It is also possible that some of the sympathetic fibers emerging from the coeliac ganglia make synaptic connections with nerve cells in the enteric plexuses rather than innervating the smooth muscle cells directly. Another obstacle to a specific interpretation of the sensitization in terms of pre- and post-ganglionic sympathetic and para-sympathetic denervation is the claim that intestino-intestinal reflexes are completed through the decentralized coeliac ganglia (13). However, evidence for the existence of such reflexes is not complete (14) (15). The experiments clearly demonstrate that destruction of all connections between the pre-aortic ganglia and the central nervous system results in only a mild degree of sensitization of the intestine to the inhibitory action of adrenalin. On the other hand, destruction of the connections between the pre-aortic ganglia and the intestine, whether these ganglia have been previously decentralized or not, causes a three- to seven-fold increase in the sensitivity of the intestine to adrenalin.

Various operations upon abdominal sympathetic nervous pathways have been performed in man for the purpose of relieving hypertension (16). The results from the dog intestine suggest that operations involving decentralization of the coeliac ganglia in man would produce less sensitization of the intestine to the inhibitory action of adrenalin than would be produced by removal of the coeliac ganglia. However, either operation would involve the destruction of most or all of the innervation of the adrenal medullae.

SUMMARY AND CONCLUSIONS

The effect of vagotomy and of sympathectomy at different levels on the sensitivity of intestinal smooth muscle to the inhibitory action of adrenalin has been determined by the use of Thiry fistulae of the jejunum in unanesthetized dogs. Wherever possible simultaneous records by the balloon-mercury-manometer method have been taken from two loops in one animal, these loops differing from each other with regard to only one set of nerves. In other cases the sensitivity of the loops in a given animal has been evaluated for a period of time before and after the denervation.

Vagotomy either has no effect on the sensitivity of jejunal smooth muscle to adrenalin or it produces a slight increase in sensitivity.

Sympathetic decentralization of the pre-aortic ganglia either has no effect or results in a less than two-fold increase in sensitivity to adrenalin.

Destruction of the nerve fibers in the mesenteric pedicle supplying an intestinal segment renders that segment several times more sensitive to adrenalin than another segment having its nervous connections with the decentralized pre-aortic ganglia intact.

The only denervations which produced marked hypersensitivity of the intestinal smooth muscle to adrenalin were those that involved sectioning of axones passing to the intestine from cell bodies located in the pre-aortic ganglia.

REFERENCES

(1) CANNON, W. B. Am. J. Med. Sci. **198**: 737, 1939.
(2) ELLIOTT, T. R. J. Physiol. **32**: 401, 1905.
(3) ELLIOTT, T. R. J. Physiol. **44**: 374, 1912.
(4) LANGLEY, J. N. AND R. MAGNUS. J. Physiol. **33**: 34, 1905.
(5) COTUI, F. W., C. L. BURSTEIN AND A. M. WRIGHT. J. Pharmacol. and Exper. Therap. **58**: 33, 1936.
(6) SHIMIDZU, K. Arch. exper. Path. u. Pharmakol. **104**: 254, 1924.
(7) YAMAKAWI, H. Jap. J. Med. Sci. **8**: 65, 1934–35.
(8) MODERN, F. S. AND C. H. THIENES. Science **83**: 522, 1936.
(9) LUCO, J. V. This Journal **120**: 179, 1937.
(10) DRAKE, M. E., F. S. MODERN, R. J. F. RENSHAW AND C. H. THIENES. Arch. internat. de Pharmacodyn. et de Therap. **63**: 224, 1939.
(11) YOUMANS, W. B. This Journal **123**: 424, 1938.
(12) MITSUDA, T. Ztschr. f. d. ges. Exper. Med. **39**: 330, 1924.
(13) KUNTZ, A. AND C. VAN BUSKIRK. Proc. Soc. Exper. Biol. and Med. **46**: 519, 1941.
(14) SHEEHAN, D. Annual Rev. Physiol. **3**: 400, 1941.
(15) YOUMANS, W. B., A. I. KARSTENS AND K. W. AUMANN. This Journal **135**: 619, 1942.
(16) WHITE, J. C. AND R. H. SMITHWICK. The autonomic nervous system. MacMillan Co., 1941, p. 323.

THE PRODUCTION OF EXPERIMENTAL POLYCYTHEMIA IN MAN BY THE DAILY ADMINISTRATION OF AMPHETAMINE SULFATE[1]

JOHN E. DAVIS AND ALBERT M. HARRIS

From the Departments of Physiology and Pharmacology, and Pathology, University of Arkansas School of Medicine, Little Rock

We have reported previously (1) the production of experimental polycythemia in dogs, rabbits, and man by the daily administration of ephedrine sulfate. The results were attributed to increased erythropoiesis induced by hypoxia of bone marrow through diminution of its blood supply, as a result of the vasoconstrictor action of ephedrine. In this work we also found that amphetamine sulfate induced polycythemia in one splenectomized and three normal dogs, but our results on rabbits were inconclusive.

The present investigation was made to determine whether amphetamine is capable of producing polycythemia in human subjects.

PROCEDURE. The subjects used for these experiments comprised four medical students and two teachers ranging from 20 to 35 years of age. All were healthy male individuals.

Several control observations were made on the blood of each individual over a period of at least 2 weeks before the drug was administered. Red cell counts, hemoglobin determinations (Hellige) and total leukocyte counts were made quite regularly, and reticulocyte percentages were estimated occasionally.

After the control period each subject took benzedrine sulfate[2] by mouth in a daily dose of 10 mgm. During the first few days blood pressure readings were made on each subject before, and at intervals after the ingestion of the drug. Observations on the blood were usually made at a definite time of day at least 20 hours after the previous daily dose of amphetamine.

RESULTS. Figure 1 shows the effect of the daily oral administration of amphetamine sulfate upon the red blood cell counts of 5 normal human subjects. It will be seen that the erythrocyte numbers of all 5 subjects were increased significantly (12 to 15 per cent) within 1 to 2 weeks after the commencement of drug administration. Hemoglobin percentages (not shown) were increased proportionately. Total leukocyte counts remained fairly constant, showing no variation in the same direction as red cell changes. The very few estimations of reticulocyte percentages showed no significant changes, although there was a suggestion of increased percentages of reticulocytes after the red cell counts had been increased. Upon cessation of amphetamine ingestion, the red cell counts returned slowly to normal, over a period of 6 to 14 days.

---

[1] Research paper no. 528, journal series, University of Arkansas.

[2] The benzedrine sulfate was courteously supplied by Smith, Kline & French Laboratories, of Philadelphia, Pa.

One subject out of the six receiving benzedrine did not show any change in his erythrocyte number, which remained quite constant around a value of about 6 million.   The data on this subject are not shown in the figure.

Blood pressure was increased uniformly at 1 to 4 hours after benzedrine in 4 of the 6 subjects, during the first few days of drug ingestion.   Diastolic pressure, particularly, was observed to be increased by about 6 mm. on the average (not shown).   The single subject who did not develop polycythemia also showed no uniform blood pressure response to amphetamine.
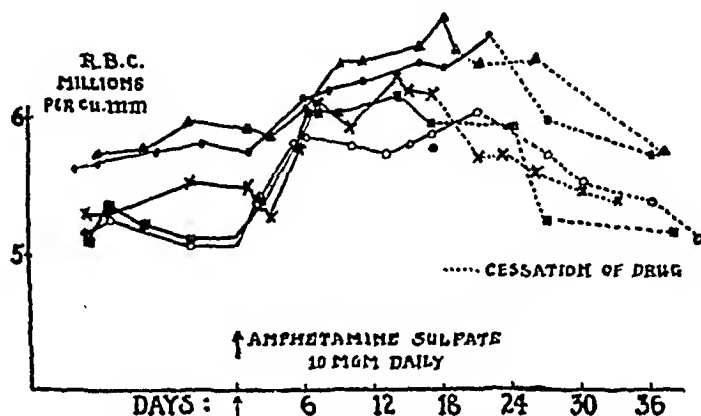
Fig. 1. Red cell counts in five normal human subjects following amphetamine administration

DISCUSSION.   Ehrich and Krumbhaar (2) observed an erythrocytosis accompanied by leukocytosis in rats which were given daily doses of 2 to 80 mgm. benzedrine sulfate.   Ehrich, Lewy and Krumbhaar (3) found that doses of 2 to 10 mgm. per kgm. daily eventually caused macrocytic anemia in dogs and other species, but that daily doses of 1 mgm. per kgm. or less were harmless to dogs even when given over a prolonged period of time.   Schube, Raskin and Campbell (4) gave daily doses of 10 mgm. of amphetamine sulfate, orally, to 6 physically normal human patients for 30 days.   They followed the blood picture in these patients at weekly intervals, but detected no significant changes.

Our strongest reason for believing that the elevated erythrocyte numbers observed in our experiments are due to increased erythropoiesis is to be found in the slow development of polycythemia (fig. 1) and the slow recovery from the same after discontinuation of the benzedrine.   The time relationships correspond generally with those involved in the production of polycythemia by exposure to low atmospheric pressure (5).

This delay in production and recovery from polycythemia also argues against the possibility that our results might be due to blood concentration or to contraction of blood reservoirs.   The relative constancy of the total leukocyte counts observed in these experiments constitutes additional evidence against the possibility of concentration of the blood.   Our previous finding that splenectomized dogs develop polycythemia from the continued administration of benzedrine or ephedrine just as readily as normal animals (1) also suggests that

blood reservoirs probably are not concerned in the development of this polycythemia.

The most likely explanation of the mechanism by which benzedrine causes polycythemia seems to rest on the assumption that the drug increases hemopoiesis by virtue of its vasoconstrictor action, i.e., causing a diminution of blood supply to the bone marrow and local hypoxia of that tissue. This explanation also seems valid for, and may be viewed as supported by, our recent finding that other vasopressor drugs (epinephrine and posterior pituitary) in appropriate doses are capable of producing polycythemia (6).

We cannot definitely explain why one subject, in the group of six, failed to develop polycythemia. We could not observe any uniform blood pressure change following his early daily doses of benzedrine, and on the basis of our theory, this fact may account for his failure to develop polycythemia, although it is not inconceivable that a selective vasoconstriction might occur without involving any change of general blood pressure.

It will be noted (fig. 1) that the average increases in red cell counts following benzedrine amounted to about 0.75 of a million. We wish to emphasize that the observations on the blood were usually made at definite hours of the day, and at least 20 hours after the daily dose of amphetamine. In a few experiments we noted no immediate change in the red cell count 2 to 4 hours after the oral ingestion of 10 mgm. of the drug.

We do not know whether a larger dose of benzedrine or a different route of administration would cause a greater polycythemia, or whether the observed polycythemia would persist with more prolonged administration of the drug.

CONCLUSIONS

The daily oral administration of amphetamine sulfate (10 mgm.) caused a significant increase in the erythrocyte numbers of 5 out of 6 normal human subjects within 6 to 12 days.

The results are explained by assuming that amphetamine increases hemopoiesis by causing a local hypoxia of bone marrow through a diminution of the blood supply to this tissue.

REFERENCES

(1) DAVIS. This Journal **134**: 219, 1941.
(2) EHRICH AND KRUMBHAAR. Ann. Int. Med. **10**: 1874, 1937.
(3) EHRICH, LEWY AND KRUMBHAAR. Am. J. Med. Sci. **198**: 875, 1939.
(4) SCHUBE, RASKIN AND CAMPBELL. New England J. Med. **216**: 922, 1937.
(5) DAVIS. This Journal **127**: 322, 1939.
(6) DAVIS. Fed. Proc. **1**: 19, 1942.

THE EFFECT OF LYMPHATIC BLOCK ON BILE RESORPTION IN OBSTRUCTIVE JAUNDICE[1]

B. G. P. SHAFIROFF, HENRY DOUBILET, W. F. RUGGIERO, A. P. PREISS
AND CO TUI[2]

From The Laboratory of Experimental Surgery, New York University College of Medicine

In a previous report from this laboratory (1) it was shown that as the intra-biliary pressure was experimentally increased above the normal secretory pressure of the liver, the bilirubin level rose in both lymph and blood, but that the blood level was significantly and consistently lower. The present report represents a study of bilirubin resorption in obstructive jaundice after blockage of the lymphatic channels by ligation of the thoracic lymph duct, thus limiting absorption to the blood stream.

Twenty-three healthy dogs ranging in weight from 12 to 15 kilos were operated upon under sterile conditions (group I). Through a transverse incision over the fifth rib, the thoracic lymph duct was ligated in the chest close to the diaphragm after the manner recommended by Lee (2). Through an abdominal incision, the common bile duct was isolated and severed between sutures. The gall bladder was removed in each case and, whenever present, accessory bile ducts leading to the duodenum were ligated. Pre-operative and post-operative samples of blood were taken at arbitrary intervals for bilirubin determination by the quantitative van den Bergh method. On the third post-operative day, the dogs were anesthetized by the intravenous injection of pentobarbital sodium, the abdomen was opened and the intraductal pressure measured with a water manometer. The cisterna chyli and thoracic lymph duct were isolated and all collateral lymphatics which might have re-established the continuity of the lymph circulation were sought for by careful dissection with or without the aid of methylene blue injected as a tracer. A final sample of blood as well as of urine was taken for quantitative bilirubin determinations. Before the animal was sacrificed a lobe of liver was removed in its distended state and sections were made for microscopic study.

This experiment was controlled in two ways. In one group of seven dogs, obstructive jaundice was produced by ligation of the common bile duct combined with cholecystectomy but without lymph obstruction (group II). In the second control group of three dogs, the thoracic lymph duct only was obstructed (group III). The three types of experiments are illustrated graphically in figure 1. In each series, bilirubin determinations on the blood, lymph and urine were done.

RESULTS. *Group I.* Simultaneous obstruction of the main lymph duct and the common bile duct was followed by edema in the retroperitoneal space along the para-aortic and para-caval planes. The edema fluid was yellowish green,

the intensity of the coloring apparently dependent on the concentration of the absorbed bile pigments. The cisterna chyli was markedly distended in all the animals examined and was found ruptured in three. In four dogs bile-stained fluid was present in the peritoneal cavity. All the intra-abdominal lymph glands were enlarged, edematous, and bile-stained. The visceral organs, as well as the intestinal tract, aside from being bile-stained, showed no gross edema or other changes ascribable to lymphatic block.

Total lymphatic block was achieved in five dogs of group I (table 1). In four of these animals, the common bile duct was found collapsed instead of distended and the intraductal pressure varied from 86 to 128 mm. water. Although the
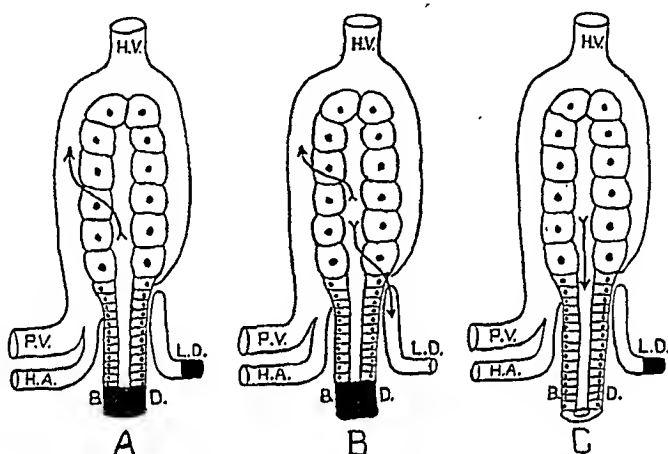
Fig. 1. Diagrammatic drawings of hepatic lobule showing direction of flow in each group of experiments. Portal vein (*P.V.*) and hepatic artery (*H.A.*) supply blood to the sinusoid which surrounds the liver lobule. The blood passes out through the hepatic vein (*H.V.*). Bile flows from hepatic lobule through bile duct (*B.D.*). The lymphatic duct (*L.D.*) originates alongside the bile duct. (A) group I, common bile duct and thoracic lymph duct obstructed; (B) group II, common bile duct obstructed; (C) group III, thoracic lymph duct obstructed.

common bile duct failed to dilate in these dogs, no accessory biliary channels leading to the intestine could be traced. In the fifth dog, however, the bile duct was markedly dilated, and measurement revealed a biliary pressure of 332 mm. of water. Bilirubin appeared in significant amounts in the blood of all five animals within two hours after operation and 65 hours later reached an average concentration of 7.04 mgm. per cent. Samples of lymph and urine obtained at the same time as the final blood sample showed average values for bilirubin of 6.99 mgm. per cent and 7.2 mgm. per cent respectively.

In the other eighteen dogs of group I (table 2), collateral lymph circulation developed from a point proximal to the severed thoracic duct, re-establishing continuity with either the distal section of the thoracic duct or the right lymph duct. Direct communication with a thoracic vein by one or more lymphatics was found in six animals. In four, the collaterals arose in the abdomen from the cysterna chyli, ascended high along the posterior chest wall, and emptied

into the distal end of the thoracic duct. In most cases, the collateral lymphatics were insufficient to maintain normal circulation of lymph as was shown by the distention of the thoracic duct and the occurrence of retroperitoneal lymph-

TABLE 1

Biliary obstruction with obstruction of the thoracic lymph duct

DOG NO.	BILIRUBIN CONCENTRATION FOLLOWING OBSTRUCTION IN MILIGRAMS PER 100 CC.										SECRETORY PRESSURE OF BILE MM. H₂O 65 HRS.
	Blood								Lymph	Urine	
	2 hrs.	4 hrs.	8 hrs.	15 hrs.	25 hrs.	35 hrs.	45 hrs.	65 hrs.	65 hrs.	65 hrs.	
1	0.53		1.87	2.15	4.61	5.00	6.86	7.00	8.24		122
2	0.43		2.38	2.86	3.16		5.18	7.54	8.00		104
3	0.00		1.27	3.15	4.10	5.68	6.24	6.76	6.32	3.9	332
4	0.31	0.84	1.41	3.20	3.63	4.12	5.83	7.23	5.40	8.3	128
5	0.52	1.02	1.34	2.06	2.34	3.82	5.29	6.70	7.02	9.6	86
Average								7.04	6.99	7.2	154

TABLE 2

Biliary obstruction with partial obstruction of the thoracic lymph duct

DOG NO.	BILIRUBIN CONCENTRATION FOLLOWING OBSTRUCTION IN MILLIGRAMS PER 100 CC.										SECRETORY PRESSURE OF BILE MM. H₂O 65 HRS.
	Blood								Lymph	Urine	
	2 hrs.	4 hrs.	8 hrs.	15 hrs.	25 hrs.	35 hrs.	45 hrs.	65 hrs.	65 hrs.	65 hrs.	
6	0.10	0.14	0.23	0.81	0.90	0.95	0.95	1.84	3.12	6.2	312
7	0.21		1.20		2.08	2.34	2.48	2.48	2.44	4.0	298
8	0.18		0.33	0.47	1.76	2.24		1.76	2.06	6.0	360
9	0.00	0.09		1.28	2.53	3.86	4.78	4.86	4.80	9.5	412
10	0.37		0.59	0.68	1.28	1.24	1.36	2.88	2.76	6.4	374
11	0.00	0.82	1.51		2.46	3.85	4.88	5.45	5.95	8.9	·284
12	0.26	0.50	0.69	1.12	2.23	3.43	4.18	5.80	4.68		186
13	0.57		1.14	1.43	1.72	2.16	3.29	4.53	3.69	2.0	304
14	0.20		1.32	1.32	2.85	3.14	4.15	5.00	6.64	9.3	214
15	0.00	0.45		0.66	1.06	1.51		3.10	3.32	6.6	194
16	0.92		1.26	2.24		2.47		4.64	4.83	4.1	208
17	0.41		0.97	1.34	1.87	2.23	3.06	4.20	5.08	11.0	184
18	0.33	0.44		0.73	1.00	0.95		3.30	3.26	6.3	218
19	0.00	0.80		1.65	2.78	3.85		5.05	5.56	4.1	
20	0.21		0.28	0.68	1.43		2.84	3.92	3.76	6.2	
21	0.86	0.98	1.44	1.84	2.43	3.88	4.23	4.47	5.12		
22	0.00	0.00	1.81	3.30	3.83	4.61	4.80	5.21	3.82	5.6	
23	0.15	0.81	1.12	1.61	1.84	3.14	3.61	4.81	2.61	5.8	406
Average								4.06	4.08	6.3	282

edema. The common bile duct was dilated in all dogs of this series, with an average figure of 282 mm. of water for the secretory pressure in the ducts. Two hours after operation, bilirubin was present in the blood of thirteen of the

eighteen animals. At the end of the experiment, the average concentration of bilirubin in the blood was 4.06 mgm. per cent, in the lymph 4.08 mgm. per cent, and 6.3 mgm. per cent in the urine. In seven animals, the bilirubin in the lymph exceeded that of the blood, in five it was significantly less, and in six others the blood and lymph were approximately equal.

Microscopic studies of liver sections from group I showed varying amounts of central necrosis, hepatic parenchymal derangement, and hepatic cell necrosis. Although many of the bile canaliculi were markedly distended, rarely was one

TABLE 3

Control dogs of group II with biliary obstruction only

DOG NO.	BILIRUBIN CONCENTRATION FOLLOWING OBSTRUCTION IN MILLIGRAMS PER 100 CC.										SECRETORY PRESSURE OF BILE MM. H₂O 65 HRS.
	Blood								Lymph	Urine	
	2 hrs.	4 hrs.	8 hrs.	15 hrs.	25 hrs.	35 hrs.	45 hrs.	65 hrs.	65 hrs.	65 hrs.	
24	0.00	0.21	0.24	0.35	0.68	0.86	1.14	2.15	2.68	4.1	340
25	0.00	0.00	0.21	0.51	0.64	1.00	1.64	1.83			
26	0.08	0.27	0.27	1.46	2.54	3.26	3.45	4.08	2.49	6.5	360
27	0.00	0.39	0.49	0.59	1.38	1.49	1.64	1.87			
28	0.00	0.00	0.31	1.68	2.86	3.08	2.87	3.00			
29	0.00	0.14	0.19	0.59	0.85	1.27	1.46	2.06			282
30	0.00	0.16	0.24	0.38	0.92	1.46	1.67	1.93	3.04	5.8	194
Average..								2.41	2.73	5.4	294

TABLE 4

Control dogs of group III with thoracic lymph duct obstruction only

DOG NO.	BILIRUBIN CONCENTRATION FOLLOWING OBSTRUCTION IN MILLIGRAMS PER 100 CC.										SECRETORY PRESSURE OF BILE MM. H₂O 65 HRS.
	Blood								Lymph	Urine	
	2 hrs.	4 hrs.	8 hrs.	15 hrs.	25 hrs.	35 hrs.	45 hrs.	6 5 hrs.	65 hrs.	65 hrs.	
31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	114
32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	108
33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	92
Average..								0.00	0.00	0.00	104

found ruptured. Lymph spaces and lymphatic capillaries could not be identified within the liver lobule or in relation to the hepatic sinusoids or liver cords, but lymphatic vessels in the periportal connective tissue were often distended.

Group II. A small amount of bile-stained fluid was found in the peritoneal cavity of each dog subjected only to obstructive jaundice. The liver was distended and deeply coloured as were the other abdominal viscera. Although the lymphatics were normal in appearance, they were easily distinguished and traced because of the bile pigment in the lymph. The intrabiliary pressure

ranged from 194 to 360 mm. (average 294) water (table 3). Bilirubin appeared in the blood about four hours after operation and reached an average concentration of 2.41 mgm. per cent in the last sample. In three cases, lymph was aspirated from liver lymphatics in the gastrohepatic omentum and was found to contain an average of 2.73 mgm. per cent of bilirubin. Bile was present in all urine specimens examined. Liver sections showed varying amounts of cell necrosis and derangement of the hepatic parenchyma.

Group III. The animals with thoracic lymph duct obstruction alone also developed retroperitoneal edema but this was not as marked as in group I. In all three dogs the thoracic duct and its tributaries were distended and contained a clear lymph. Careful search at autopsy showed that complete lymphatic block had been attained in these animals. Bilirubin was not present in either the serum, lymph or urine. The common duct pressure was 114, 108 and 92 mm. of water, with an average of 104 mm. (table 4). Microscopic studies of the liver sections yielded no significant evidence of intralobular lymphatics.

EXPERIMENTAL DATA. The following observations also deserve mention. In the animals with total lymphatic block, a low ductal pressure was found. In the controls with only thoracic lymph duct obstruction, a low intraductal pressure was also obtained. In those animals in which a collateral lymph circulation developed after ligation of the lymph duct, the intraductal pressure was approximately the same as in the dogs subjected to biliary obstruction alone.

A comparison of the average concentration of blood bilirubin at the end of the experiments in each group revealed the following figures: (1) biliary obstruction only, 2.41 mgm. per cent, (2) biliary obstruction with partial lymphatic obstruction, 4.07 mgm. per cent, and (3) biliary obstruction with complete lymphatic block, 7.06 mgm. per cent of bilirubin.

In four of the five animals in which complete lymphatic block was achieved, a noteworthy point was the lack of distention of the bile ducts due to the diminished intrabiliary pressure. This diminished intrabiliary pressure was not associated with obstruction of the bile duct since simple obstruction did not diminish the secretory pressure of the liver in the dogs of group II but was associated with complete lymphatic block, a procedure which in itself (group III) served to reduce the secretory pressure. The mechanism responsible for the reduction of the secretory pressure of the liver by complete lymphatic block may have been due to either hepatic cell injury or to an increased rate of escape of secreted bile into the intralobular hepatic veins. A third possibility was that the lymphatic block resulted in swelling of the hepatic cells, which produced pressure block of the bile capillaries and so decreased the secretory pressure of the bile (3).

DISCUSSION. The amount of bilirubin resorbed by the blood in experimental jaundice has been investigated by many workers. Bollmann, Sheard and Mann (4) noted the marked rapidity of the onset of jaundice and bilirubinemia after ligation of the common bile duct and removal of the gall bladder. Snell (5) and his associates made a detailed analysis of the changes in the blood and

urine of jaundiced dogs. In their series, the greatest rise in blood bilirubin occurred from the third to the fifth post-operative day and fell as the obstruction became chronic.

Bloom (6) studied the relationship of the lymphatics to obstructive jaundice and noted the appearance of bilirubin within 48 minutes in the lymph and within two hours in the blood. In this investigation, complete obstructive jaundice with cholecystectomy and lymphatic stoppage appeared to produce a more rapid onset and higher degree of bilirubinemia than that obtained by ligation of the common bile duct and cholecystectomy.

The reason for the increased concentration of bilirubin in the blood when the lymphatic flow from the liver is blocked, is not clear. Several possibilities may be considered which involve such factors as renal damage, diminished excretion of urine, or dehydration with hemoconcentration. None of these factors were investigated.

In obstructive jaundice the bile may be resorbed either by the intralobular radicals of the hepatic veins or by the liver lymph. The importance of the absorptive rôle of the lymphatics of the liver has been the subject of controversy. Whipple and King (7), Bollmann et al. (8), Mendel and Underhill (9), all have claimed that the "lymphatic apparatus takes no essential or active part in the absorption of bile pigments." This view is not shared by the authors. Neither is it our view that the lymphatics are the primary resorptive pathways or the only absorptive channels as proposed by Bloom (6), Fleischl (10), Kufferath (11), Eppinger (12) and Harley (13).

It is our belief that both the blood and the lymph systems take part in the absorption of bile during jaundice. Previously, we demonstrated that absorption depended on intrabiliary pressure, as a result of which, bilirubin appeared first in the lymph and later in the blood (1). However, the concentration of bilirubin in the blood tended to attain and maintain a constant level probably because of the regulatory effect of the kidneys, while in the lymph its concentration depended directly on biliary pressure. Although we have been unable to secure total lymphatic block in the majority of animals, as was also the experience of Blalock (14), the results in five animals with complete lymphatic block showed that the entire absorptive function can be taken over by the blood when and if the lymphatic pathway is completely blocked.

SUMMARY

Biliary obstruction with complete block of the thoracic lymph duct produced a high concentration of bilirubin in the blood at the end of 65 hours. This concentration was much greater and more rapid in onset than that obtained by partial lymphatic block, and greater still than that obtained by simple biliary obstruction.

Complete stoppage of lymph flow through the thoracic duct markedly reduced the secretory pressure of bile in the extra hepatic bile ducts. When biliary obstruction was produced in the presence of complete lymphatic block, the bile ducts did not dilate in four out of five experiments.

REFERENCES

(1) SHAFIROFF, DOUBILET AND RUGGIERO. Proc. Soc. Exper. Biol. and Med. **42**: 203, 1939.

(2) LEE. Bull. Johns Hopkins Hosp. **33**: 21, 1922.

(3) IVY. Personal communication.

(4) MANN AND BOLLMAN. J. Lab. and Clin. Med. **10**: 540, 1925.

(5) SNELL, GREENE AND ROWNTREE. Arch. Int. Med. **40**: 471, 1927.

(6) BLOOM. Bull. Johns Hopkins Hosp. **34**: 316, 1923.

(7) WHIPPLE AND KING. J. Exper. Med. **13**: 115, 1911.

(8) BOLLMAN, SHEARD AND MANN. This Journal **80**: 461, 1927.

(9) MENDEL AND UNDERHILL. This Journal **14**: 252, 1905.

(10) FLEISCHL. Ber. über d. Verhand der K. Sachissen Gesellschaft **26**: 42, 1874.

(11) KUFFERATH. Arch. f. Anat. u. Physiol. 291, 1893.

(12) EPPINGER. Beitr. z. path. Anat. u. z. Allg. Path. **31**: 230, 1902.

(13) HARLEY. U. Arch. f. Anat. u. Physiol. Physiol. Abteilung 291, 1893.

(14) BLALOCK, ROBINSON, CUNNINGHAM AND GRAY. Arch. Surg. **34**: 1049, 1937.

INFLUENCE OF ADRENALECTOMY UPON THE RATE OF GLUCOSE ABSORPTION FROM THE INTESTINE

WILLIAM G. CLARK AND EATON M. MacKAY

From the Scripps Metabolic Clinic, La Jolla, California

There has been disagreement as to whether or not adrenalectomy has a specific effect upon the absorption of glucose from the intestinal tract. The controversy had its origin in the contention of Verzar (1, 2) that there is a failure in the selective absorption of metabolizable sugars following adrenalectomy (3). The work serving as a basis for this conclusion (3) as well as the experiments of Marrazzi (4) utilized intestinal loops. The objections inherent in this method are of such a nature that their applicability to this particular problem must be seriously questioned. The present study is not concerned with absorption from intestinal loops and data obtained in this manner have not been considered. Rather we were interested in observing the influence of adrenalectomy upon the absorption rate for glucose in intact, unanesthetized animals. Cori's technique (5) was used for this purpose. It must be realized that although this method avoids the objections to the use of isolated intestinal loops it introduces new difficulties such as the influence of the stomach emptying time on the absorption from the gut (6). The influx into the stomach of a large volume of concentrated glucose solution during a few seconds undoubtedly affects intestinal motility and in other ways influences absorption. At any rate, the results obtained with such a method are far different from those observed under conditions of voluntary feeding (7) and must be considered in the light of the *special conditions* which they represent.

Cori's procedure (5) was followed with modifications which have been described (8). The analytical methods were the same as those which have been used before (7) except that sodium fluoride was used to inhibit glucolysis and chloride (9) and sodium (10) were determined in some cases by standard methods. Zinc sulfate was used in place of sodium fluoride in these cases.

The adrenalectomies were performed under ether anesthesia, the glands being removed from a single skin incision on the back through a small lumbar incision of the muscles on either side. This operation has been performed many thousands of times, requires a total period of less than two minutes, and the rats recover from the anesthetic showing no ill effects of the operation, within five to ten minutes later. Control animals were operated upon in the same manner but the adrenals were left intact.

When glucose absorption was measured very shortly (24 hrs.) after adrenalectomy, before it might be affected by failure of the appetite or other changes secondary to the adrenalectomy but when the effects of the operation might well still be present, the data (table 1, expt. 1) show quite clearly that there is a decrease in the rate of glucose absorption from the gastrointestinal tract. This

may be due to an impairment of the mechanism concerned in the absorption of glucose by the intestinal mucosa as Laszt and Verzar (11) believe or secondary

TABLE 1

The influence of adrenalectomy on the rate of absorption of glucose from the intestine

EX-PERI-MENT NO.	ABSORP-TION PERIOD	NO. ANI-MALS PER GROUP	CONTROLS				ADRENALECTOMIZED				DECREASE IN AB-SORPTION RATE	NaCl SUPPLE-MENT
			Body weight		Body surface	*Absorp-tion coeffi-cient	Body weight		Body surface	*Absorp-tion coeffi-cient		
			At opera-tion	When tested			At opera-tion	When tested				
	hrs.		gm.	gm.	sq. dm.	mgm.	gm.	gm.	sq. dm.	mgm.	per cent	
1	1	4	142		3.1	116	142		3.1	86	26	0
	2	4	141		3.1	96	141		3.1	73	24	0
	3	4	140		3.1	87	132		2.9	67	23	0
	4	4	141		3.1	80	139		3.0	64	20	0
	6	4	141		3.1	77	141		3.1	51	34	0
2	2	10	172	177	3.6	105	206	161	3.4	81	23	0
3	2	10	198	194	3.8	96	204	190	3.8	95	0	+
4	2	6	162	152	3.2	91	160	144	3.0	76	16	0
5	2	6	157	150	3.1	89	162	148	3.1	93	0	+

* Mgm. per sq. dm. body surface per hour for the period.

Exp. 1. Averages for four female rats of approximately the same age in each group. Fasted for 24 hours from the stock diet, operated and fasted for 24 hours more before testing. Two milliliters of 43.8 per cent glucose solution per sq. dm. body surface was given each rat at the beginning of the absorption period.

Exp. 2. Female rats given the stock diet and a solution of 0.5 per cent NaCl plus 0.2 per cent sodium bicarbonate for 14 days after adrenalectomy. They were fasted and allowed only tap water for 36 hours prior to testing the absorption rate. For the latter 1 ml. 30 per cent glucose per sq. dm. body surface was administered. The adrenalectomized rats were prostrated for a short time but there was no fall in body temperature.

Exp. 3. Exactly like experiment 2 except that the solution of sodium salts was offered during the fasting period and removed only when the glucose was given.

Exp. 4. Male rats on the stock diet tested 10 days after operation. During this period they were given 1 ml. water per sq. dm. body surface per day twice daily by stomach tube and tap water was offered ad lib. They were fasted for 36 hours prior to testing. The stomach and intestinal contents were examined separately. It was found that the controls had 320 mgm. and the adrenalectomized rats 288 mgm. glucose per sq. dm. body surface available for absorption during the two hour period. The dose of glucose given was 2 ml. 25% per sq. dm. body surface.

Exp. 5. Exactly the same as experiment 4 except that both groups were given a solution of 1.0 per cent NaCl plus 0.2 per cent sodium bicarbonate by stomach tube in place of the water. This was continued until testing. In the controls it was found that 302 and in the adrenalectomized rats 338 mgm. glucose per sq. dm. body surface had entered the intestine and was hence available for absorption during the two hour period.

either to changes in the stomach emptying time and hence the amount of glucose available for absorption (6) or a result of the alterations in salt metabolism which

REFERENCES

(1) VERZAR, F. AND E. J. McDougALL.   Absorption from the intestine.   London, Long-
     mans, Green & Co., 1936.
(2) VERZAR, F.   Die function der nebennierenrinde.   Basel, B. Schwabe & Co., 1939.
(3) WILBRANDT, W. AND L. LENGYEL.   Biochem. Ztschr. 267: 204, 1933.
(4) MARRAZZI, R.   This Journal 131: 36, 1940.
(5) CORI, C. F.   J. Biol. Chem. 66: 691, 1925.
(6) MACKAY, E. M., H. C. BERGMAN AND R. H. BARNES.   Proc. Soc. Exper. Biol. and Med.
     32: 323, 1934–35.
(7) MACKAY, E. M. AND W. G. CLARK.   This Journal 135: 187, 1941.
(8) MACKAY, E. M. AND H. C. BERGMAN.   J. Biol. Chem. 101: 453, 1933.
(9) VAN SLYKE, D. D.   J. Biol. Chem. 58: 523, 1923–24.
(10) BUTLER, A. M. AND E. TUTHILL.   J. Biol. Chem. 93: 171, 1931.
(11) LASZT, L. AND F. VERZAR.   Biochem. Ztschr. 292: 159, 1937.
(12) CORI, G. T. AND C. F. CORI.   J. Biol. Chem. 73: 555, 1927.
(13) DEUEL, H. J. JR., L. F. HALLMAN, S. MURRAY AND L. T. SAMUELS.   J. Biol. Chem.
     119: 607, 1937.
(14) RUBIN, M. I. AND E. T. KRICK.   Proc. Soc. Exper. Biol. and Med. 31: 228, 1934.
(15) ALTHAUSEN, T. L., E. M. ANDERSON AND M. STOCKHOLM.   Proc. Soc. Exper. Biol. and
     Med. 40: 342, 1939.
(16) HAMAR, N.   Pflüger's Arch. 244: 157, 1940.
(17) SCHECHTER, A. J., M. K. CARY, A. L. CARPENTIERI AND D. C. DARROW.   Am. J. Dis.
     Child. 46: 1015, 1933.
(18) GILMAN, A.   This Journal 108: 662, 1934.
(19) DARROW, D. C. AND H. YANNET.   J. Clin Investigation 14: 266, 1935.

THE INFLUENCE OF THE SERUM BROMIDE CONCENTRATION UPON THE DISTRIBUTION OF BROMIDE ION BETWEEN SERUM AND SPINAL FLUID[1]

EVERETT G. WEIR

From the Department of Physiology, Howard University, Washington, D. C.

Experimental studies of the distribution of bromide ion between serum and cerebrospinal fluid in man and in dogs have led to general acceptance of the presence, between these fluid compartments, of a barrier which prevents free passage of bromide ion into the spinal fluid. Proof of the existence of this barrier is afforded by the many observations that the distribution ratio $\dfrac{[Br^-]_s}{[Br^-]_{sp.\,fl.}}$ is greater than 1.

In spite of this qualitative agreement among all workers, it is clear, from the available evidence, that quantitative differences exist. Thus, whereas in man the value of the ratio, R, is found to vary but little from 3 (1) (2) (3), in dogs ratios varying from 1.2 to 1.5 are usually found (4) (5) (6).

In seeking an explanation of the marked variation of R in man and dog, the question of the possible influence of the serum bromide level arises. In man the barrier permeability to bromide ion has been studied principally under conditions of low serum bromide concentration while in dogs levels which many clinicians consider toxic to man are commonly maintained. Furthermore in isolated instances in which R has been studied in patients who, according to clinical standards, are severely intoxicated with sodium bromide, the observation has been made that the value of the distribution ratio tends to be low (2).

In view of these considerations it appeared worth while to study systematically the effect of alterations in serum bromide concentration upon the value of R.

METHODS. Dogs were used exclusively in this study. In most instances sodium bromide was administered intravenously in single doses except when very high serum bromide levels were desired. In those cases repeated daily doses were given. On a few occasions the salt was administered by mouth. At least 24 hours were allowed for equilibrium to be established after the final administration of sodium bromide.

Spinal fluid was obtained by cisternal puncture under ether anesthesia. Blood was withdrawn under oil from the saphenous vein and centrifuged. The serum was used for analysis. Bromide and water determinations on the serum and spinal fluid were performed according to the methods previously described for serum (7).

RESULTS. The sodium bromide was administered in such quantities that the dogs studied fell into three groups. In group I (table 1) the serum level of

TABLE 1

Showing the distribution ratio of bromide between serum and spinal fluid at intermediate serum bromide levels

DOG	BROMIDE MM/KGM. H$_2$O		H$_2$O GM./KGM.		Br(s): Br (SP. FL.) (R)
	Serum	Spinal fluid	Serum	Spinal fluid	
1	22.5	19.1	924.0	987.0	1.18
2	25.3	19.8	916.0	988.0	1.28
5	25.9	17.8	910.0	989.0	1.45
6	32.3	26.7	928.0	988.0	1.21
7	33.2	29.1	927.0	987.0	1.14
8	33.6	25.1	916.0	987.0	1.34
9	33.9	28.4	925.0	991.0	1.19
10	34.2	27.9	930.0	989.0	1.22
11	37.2	27.2	925.0	987.0	1.37
12	38.6	33.3	921.0	984.0	1.16
13	41.4	34.8	932.8	989.8	1.19
14	42.2	34.2	924.0	987.0	1.23
15	45.7	38.5	902.0	987.5	1.19

TABLE 2

Showing the distribution ratio of bromide between serum and spinal fluid at low serum bromide levels

DOG	BROMIDE MM/KGM. H$_2$O		H$_2$O GM./KGM.		Br(s): Br (SP. FL.) (R)
	Serum	Spinal fluid	Serum	Spinal fluid	
16	1.50	0.91	934.0	990.0	1.65
17	1.61	0.81	931.0	990.0	1.98
18	1.73	1.01	922.0	990.0	1.71
19	5.0	2.63	920.0	988.0	1.90
20	6.5	3.4	920.0	990.0	1.89
21	6.76	4.05	917.0	989.5	1.67
22	6.8	3.74	923.	988.0	1.82
23	11.8	7.26	916.5	989.5	1.65

TABLE 3

Showing the distribution ratio of bromide between serum and spinal fluid at high serum bromide levels

DOG	BROMIDE MM/KGM. H$_2$O		H$_2$O GM./KGM.		Br(s): Br (SP. FL.) (R)
	Serum	Spinal fluid	Serum	Spinal fluid	
24	73.1	68.4	916.0	991.4	1.07
25	74.8	72.0	920.0	989.5	1.04
26	83.2	75.9	957.8	991.1	1.09
27	85.3	80.7	907.0	984.0	1.06
28	86.0	79.1	916.0	986.0	1.09
29	96.0	81.6	955.7	990.3	1.17

sodium bromide was in the range generally employed for bromide studies in dogs, namely, 20 to 50 mM Br/kgm. H_2O; in group II (table 2) the serum bromide level was low—1 to 10 mM Br/kgm. H_2O and in group III (table 3) the level was high—70 to 95 mM Br/kgm. H_2O.

Inspection of these data reveals that in the animals comprising group I the value of the distribution ratio varied between the minimum value of 1.14 and the maximum value of 1.45; the average for all animals in this group is 1.25. The ratio 1.14 obtained for dog 7, besides being the lowest found in the present series, is the lowest we have seen reported in the literature at similar bromide concentrations. It may therefore be considered an unusual finding. Within this group of animals there is no suggestion of a regular relationship between the value of R and the serum bromide level.

TABLE 4

Illustrating the time necessary to reach equilibrium between serum and spinal fluid

DOG	SAMPLE	BROMIDE mM/KGM. H_2O	$\dfrac{[Br^-]_S}{[Br^-]_{SP. FL.}}$	TIME (HOURS)
3	Serum 1	39.6	1.53	9
	Serum 2	37.2	1.33	13
	Serum 3	37.7	1.30	15
	Spinal fluid 1	25.9		
	Spinal fluid 2	27.9		
	Spinal fluid 3	29.0		
4	*Serum	27.5		
	Spinal fluid 1	19.0	1.45	10
	Spinal fluid 2	20.0	1.37	13
	Spinal fluid 3	21.05	1.30	16
	Spinal fluid 4	20.9	1.31	20

* Average concentration for samples 1, 2, 3, 4

Compared with the animals of group I, the value of the distribution ratio in the dogs of group II is quite high, the average value being 1.78. It is noteworthy that even the lowest value of R obtained in this group is considerably higher than the highest value found in group I. Nevertheless among the dogs in group II, as was the case in group I, the value of the ratio varies in a manner apparently unrelated to the serum bromide level.

The effect of massive doses of sodium bromide upon the value of R is indicated from the results obtained on the dogs of group III. In five of the six dogs studied the value of the distribution ratio is lower than even the unusually low value of 1.14 obtained in dog 7 of group I. The average value of R in these dogs is 1.09.

Early in this study experiments were conducted to determine the time required for equilibrium to be established between blood and spinal fluid. Following intravenous administration of sodium bromide, samples of blood and spinal fluid were obtained at two hour intervals and analyzed. It was soon

apparent, however, that equilibrium is reached slowly and hence, since the amount of spinal fluid one can obtain from dogs by cisternal puncture is limited, a long interval of time was allowed to elapse in later experiments before the initial samples were withdrawn. The results of two such experiments, shown in table 4, indicate that equilibrium is reached within 10 to 13 hours. These observations·agree with those reported by Wallace and Brodie who state that seven or more hours are required for attainment of equilibrium (8).

DISCUSSION. It is clear from these data that when wide variations in the level of serum bromide exist, the value of the distribution ratio $\dfrac{[Br^-]_s}{[Br^-]_{sp. fl.}}$ bears an inverse relationship to the serum bromide concentration. Recently Wallace and Brodie (8) reported that the distribution ratio of iodide ion between serum and spinal fluid increases with decreasing serum iodide concentration. Their stated opinion is that the same relationship holds for bromide and thiocyanate ions.

Many factors aside from expected individual differences may operate to mask this relationship within more limited variations in serum bromide concentrations. For example, it is a well known fact that the kidney preferentially excretes chloride ion over bromide ion. This property of the kidney together with the slow rate at which equilibrium between the blood and spinal fluid is reached might prevent a true state of equilibrium from ever being attained. Any fortuitous circumstance which might cause the kidney to increase the urinary output even temporarily would result in an alteration in the bromide to chloride ratio in the blood to which the spinal fluid might not become adjusted for a considerably longer time. If samples were drawn before this adjustment were complete only approximate equilibrium would exist and hence the value of R would be altered. However, when the distribution ratio is studied over extreme ranges in serum bromide concentration the variations in the ratio may be too great to be concealed by a temporary "dis-equilibrium."

From these data it is obvious that the difference in the value of the ratio between man and dog is not to be explained entirely upon the basis of serum bromide levels. In no instance in the dog were ratios as high as 3 obtained even when the serum bromide concentration was extremely low. Until more information is available regarding the mechanism by which spinal fluid is formed, this discrepancy must be attributed to a "species difference." Only a suggestion as to what this difference might be can be offered at this time.

Weed particularly has called attention to the dual source of the cerebrospinal fluid (9). Part of the fluid originates at the choroid plexuses and a part apparently reaches the subarachnoid spaces via the pericapillary spaces. The consensus is that the fluid from the plexus is a secretion (10). The fluid derived from the capillaries we presume to have the characteristics of an ultra-filtrate of serum although this view has been questioned recently (11). It is entirely possible that in different species, the amount of fluid contributed by each source differs. To a lesser degree, this situation undoubtedly occurs within a given species; indeed in any given animal it is not unreasonable to suppose that the

proportion of fluid from each source varies under different conditions. Unless one makes what, on the basis of available evidence, would seem to be an unjustifiable assumption that the choroid plexus and the ordinary capillaries of the brain offer the same resistance to the passage of bromide ion into the spinal fluid, it follows that the value of the distribution ratio must vary as the proportion of fluid coming from either source of spinal fluid varies. It seems that this point of view is more tangible and involves fewer assumptions than the equally plausible viewpoint that this difference between species as well as intraspecies differences is attributable to different grades of permeability of the barrier to bromide ion.

We believe that our data on the dog are most readily explained by assuming that sodium bromide alters the barrier existing between the blood and spinal fluid. The extent of alteration appears to increase with increasing concentration of bromide ion in the organism. As a result of this change, the distribution ratio approaches the ratio one would expect to find between serum and its ultrafiltrate. Whether the alteration is of such a nature as to cause a diminution in the proportion of fluid contributed by the choroid plexus where, considering all available evidence, the barrier would appear to be located or whether it changes the nature of the secretion in the direction of an ultrafiltrate cannot be decided by these data.

CONCLUSION

The distribution ratio of bromide ion between serum and spinal fluid varies inversely with the concentration of bromide in the serum. Reasons for this finding are suggested.

REFERENCES

(1) MALAMUD, W., D. FUCHS AND N. MALAMUD. Arch. Neurol. and Psychiat. **20**: 780, 1928.
(2) LOVELL, H. W. AND J. R. BROWN. Proc. Soc. Exper. Biol. and Med. **32**: 516, 1934.
(3) KATZENELBOGEN. The cerebrospinal fluid and its relation to the blood. Johns Hopkins Press, Baltimore, 1935.
(4) MASON, M. F. J. Biol. Chem. **113**: 61, 1936.
(5) WALLACE, G. B. AND B. B. BRODIE. J. Pharmacol. and Exper. Therap. **65**: 220, 1939.
(6) WEIR, E. G. AND A. B. HASTINGS. J. Biol. Chem. **129**: 547, 1939.
(7) WEIR, E. G. This Journal **127**: 338, 1939.
(8) WALLACE, G. B. AND B. B. BRODIE. J. Pharmacol. and Exper. Therap. **68**: 50, 1940.
(9) WEED, L. H. J. Med. Res. **26**: 51, 1914.
(10) FLEXNER, L. B. Physiol. Rev. **14**: 161, 1934.
(11) WALLACE, G. B. AND B. B. BRODIE. J. Pharmacol. and Exper. Therap. **70**: 418, 1940.

THE FASTING RESPIRATORY METABOLISM OF THE WHITE RAT FOR 36 HOURS FOLLOWING CONTROLLED FEEDING

CLARA L. KINGDON, IVAN L. BUNNELL AND FRED R. GRIFFITH, JR.

From the Department of Physiology, University of Buffalo, Buffalo, N. Y.

In spite of the extensive use that is made of the fasting rat for study of metabolic problems, surprisingly little has been done to define accurately the course of variation of the total respiratory metabolism following withdrawal of food. There is some information regarding oxygen consumption and respiratory quotient of the 24-hour fasting animal, and some for other isolated intervals (see Benedict and MacLeod, 1929, and Horst, Mendel and Benedict, 1930, for summaries, and their own results), but until after this work was under way, that of Wesson (1931) represented the only attempt to provide complete, continuous, hour-by-hour description of these functions during the first 44 hours of a fast. Discrepancies among these earlier data justified re-examination of the subject; and the more recent work of Werthessen (1937), though adequate in scope, has so confused the issue by the unusual nature of the results obtained, that additional evidence is even more imperative.

METHODS. *Animals and diet.* All determinations were made on male albino rats of our colony, a hardy, fertile, normal-growing Wistar strain, inbred for many generations and quite free from internal and external parasites and organic disease. The animals used for this work were the second generation on a carefully prepared diet, specially fortified with vitamins and minerals to insure complete adequacy. This consisted of whole wheat, "Klim", NaCl, $CaCO_3$, yeast and cod liver oil.

Controlled feeding. When six weeks old the animals were placed in individual cages, and at $5\frac{1}{2}$ months of age (average weight, 303 grams) were started on controlled feeding, at two periods each day: from 9 to 11 a.m. and 3 to 5 p.m. After an initial weight loss on this regime there was complete recovery and daily food-consumption records showed normal, average intake.

This method of controlled feeding makes it possible to know with some exactness when and how much the animal last ate, thus giving greater accuracy in determining the exact hours of fasting than the common procedure of counting from the time of removal of food, to which there has been constant access and which may have been last partaken of an indeterminate time previously.

Similar precaution as to feeding was previously taken by Wesson (1931) and, more recently, Werthessen (1937); and although the latter attributed to it the unusual and peculiar nature of his results, there is nothing in those of Wesson or, as will be shown later, of our own, to indicate it had any untoward or disturbing metabolic effect; a conclusion also in agreement with that of Kleiber and Smith (1940).

Apparatus. The closed-circuit metabolism apparatus described by Schwabe

114

and Griffith (1938) was used, modified to the extent that carbon dioxide, instead of being titrated, was measured by change in electrical conductivity of the $Ba(OH)_2$ solution used to absorb it, by apparatus designed and constructed in this laboratory by Mr. Richard J. Jones. This improvement upon the original model made it possible to follow CO_2 output minute by minute and thus relate it accurately to the concurrently, graphically recorded O_2 consumption.

The advantages of this apparatus are: record of the O_2 consumption is graphic and continuous and can be determined for any desired interval so that periods are regulated only by the basal state of the animal and not by limitations of the experimental apparatus; continuous activity records of the animals are obtained graphically in the record of O_2 consumption, making it possible to eliminate all experimental runs complicated by this disturbing factor. Only rigorously basal periods were used for the results reported here; these varied in length from 15 to 40 minutes.

The apparatus was calibrated originally and after any major change or replacement by addition of CO_2 or withdrawal of air at known rates and volumes; it was checked frequently and routinely by burning a jet of illuminating gas at rates of combustion approximating those of the experimental animals, as described by Bunnell and Griffith (1940). Benedict (1930, p. 175) summarizes his own experience, and undoubtedly that of all others, with attempted application of alcohol checks at rates of combustion similar to the metabolic rate of the rat as a "tour de force" capable of only occasional success. It may, therefore, not be amiss, in evaluation of our results, to call particular attention to the reliable, routine check to which our apparatus was periodically subjected.

Temperature. The effect of temperature upon metabolic rate was controlled by keeping the animals in a constant-temperature room averaging 26°C ± 2° at all times. During an experimental run the animal chamber of the metabolism apparatus was kept at 28°–30°C.

Procedure. It was desired to describe the variation in respiratory metabolism hour-by-hour for the first 36 hours of fasting. To do this two courses were open: continuous records from the last ingestion of food, without interruption or removal of the animal from the metabolism apparatus; or short period (2–4 hrs.) determinations, repeated often enough to cover the entire 36-hour interval, the animal remaining free in its cage, but without food, until the time chosen for any particular test period.

Short period determinations: This method was tried first, for technical reasons as well as from the fear that too-long confinement in the metabolism chamber with its restriction of activity and lack of water might influence the results. Theoretically, the best application of this method would have involved the use of a large number of animals so that determinations at each fasting hour would be made at constant age and weight. This was not feasible, not only because the necessary numbers were not available but also because the required preliminary training in controlled-feeding would have made it prohibitive.

The work was therefore started with four rats, when 8 months old, after having been on controlled-feeding 2½ months and with an average weight of 316 grams.

A minimum goal was set of 10 determinations for each hour, as being the least that might be expected to furnish a relatively valid average. The animals were used in rotation in order to space the fasts and prevent any cumulative effect of under-nutrition. Since this procedure was anticipated to entail considerable elapsed time before all of the required data would be accumulated, the trial periods were staggered so that, in so far as possible, variations due to increasing age and weight would be equally distributed over the whole average curve. Actually, with the unavoidable delays inherent in such protracted work, it was a year before the series was completed as far as the 30th hour of fasting. By this time the animals were 20 months old, with an average weight of 367 grams. Due to individual rates of growth, the effort to equalize the change in weight was not too successful, the averages for the 10 determinations at each hour varying between 327 to 358 grams. In addition, seasonal variations, if any (Benedict and MacLeod, 1929, p. 371) would have been introduced and unequally distributed. These factors, in retrospect, seemed sufficient to account for the irregularities of the final, average curves; their implication being even more probable from the fact that respiratory quotient, presumably less affected by age and body size than total O_2 consumption or CO_2 production, showed the least irregularity. These data were considered valuable, however, and will be referred to in confirmation of those definitively obtained by continuous records, as follows.

Continuous determinations: Each of the above four animals was at one time (when, respectively, 14, 15, 15 and 16 mos. old) observed continuously for the first 24 hours of fasting. To these four records for the first 24-hours were later added six others obtained from a new group of four animals. These were treated and trained in controlled-feeding exactly as the first group and were used for these determinations when 7 to 9 months old; two of them being used twice, when 7 and 9, and 8 and 9 months old, respectively. In addition, each animal of this second group was observed twice over the fasting interval of 25 to 36 hours, when they were 10 and 12, 12 and 13, 11 and 12, and 11 and 12 months old, respectively.

In conclusion, the final and definitive data for O_2 consumption and CO_2 production are, therefore, derived from ten continuous runs covering the first 24 hours, plus eight continuous observation periods covering the last 12 hours of a 36-hour fast. Within each of these intervals the data from hour to hour are completely homogeneous and free from any relative disturbance due to age, weight or seasonal differences. Between the two sections of the curve, joining at the 24–25 hour interval, some hiatus might be expected due to the slight difference in age composition of the two groups; actually, there was none; probably due to the fact that the average age and weight of the 1–24-hour group (11 months; 342 grams) was almost exactly the same as that of the 24–36-hour group (11.6 months; 336 grams).

RESULTS. *Oxygen consumption and carbon dioxide production.* As mentioned in the preceding section, the results giving the smoothest average curves for these two variables are those derived from ten 1–24-hour, and eight 25–36-hour

continuous runs. These are shown in table 1 and figure 1. As, also mentioned previously, these data are substantiated (for the first 30 hrs.) in every important particular, such as maximal and minimal values, percentage change and time

TABLE 1

HOURS FASTING	OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION						RESPIRATORY QUOTIENT		CALORIES PER m²/24 HRS.	
	Number of determinations	Average weight, grams	Oxygen		Carbon dioxide		Number of determinations	Average	Average	Per cent of maximum
			cc. per minute	Per cent of maximum	cc. per minute	Per cent of maximum				
1	10	342	4.83	100.0	4.74	100.0	20	0.970	954	100.0
2	10	342	4.70	97.3	4.42	93.2	20	0.925	915	95.9
3	10	342	4.69	94.1	4.33	91.3	20	0.941	915	95.9
4	10	342	4.57	94.6	4.39	92.6	20	0.942	901	94.4
5	10	342	4.61	95.4	4.34	91.5	20	0.901	882	92.5
6	10	342	4.59	95.0	4.11	86.7	20	0.928	899	94.2
7	10	342	4.53	93.8	3.89	82.1	20	0.867	879	92.1
8	10	342	4.47	92.6	3.94	83.1	20	0.841	869	91.1
9	10	342	4.42	91.5	3.62	76.4	20	0.788	855	89.6
10	10	342	4.42	91.5	3.58	75.5	20	0.795	853	89.4
11	10	342	4.42	91.5	3.44	72.6	20	0.788	831	87.0
12	10	342	4.34	89.9	3.48	73.4	20	0.788	818	85.7
13	10	342	4.28	88.6	3.51	74.1	20	0.808	812	85.1
14	10	342	4.29	88.8	3.51	74.1	20	0.774	817	85.6
15	10	342	4.14	85.7	3.35	70.7	20	0.798	780	81.7
16	10	342	4.19	86.8	3.49	73.6	20	0.807	795	83.4
17	10	342	4.20	87.0	3.32	70.0	20	0.780	789	82.7
18	10	342	4.05	83.9	3.28	69.2	20	0.798	765	80.2
19	10	342	4.00	82.8	3.08	65.0	20	0.789	748	78.4
20	10	342	4.01	83.0	3.01	63.5	20	0.763	746	78.1
21	10	342	4.07	84.3	3.12	65.8	20	0.755	763	80.0
22	10	342	4.31	89.2	3.29	69.4	20	0.754	806	84.4
23	10	342	4.16	86.1	3.06	64.5	20	0.736	791	82.9
24	10	342	4.25	88.0	3.26	68.8	20	0.774	793	83.0
25	8	336	4.17	86.3	2.95	62.2	18	0.744	777	81.4
26	8	336	4.08	84.5	2.89	61.0	18	0.744	762	79.8
27	8	336	3.95	81.8	2.79	58.8	18	0.727	738	77.3
28	8	336	3.92	81.2	2.85	60.1	18	0.697	731	76.6
29	8	336	4.06	84.1	2.96	62.4	18	0.710	761	79.8
30	8	336	3.95	81.8	2.77	58.4	18	0.721	737	77.2
31	8	336	4.05	83.9	2.94	62.0	8	0.730	760	79.6
32	8	336	3.91	81.0	2.88	60.8	8	0.713	730	76.4
33	8	336	4.18	86.5	2.96	62.4	8	0.710	780	81.7
34	8	336	4.05	83.9	2.90	61.2	8	0.718	758	79.4
35	8	336	4.01	83.0	2.78	58.6	8	0.683	747	78.3
36	8	336	3.98	82.4	2.76	58.2	8	0.703	742	77.8

relations, by the results of an equal number of short-period (2–4 hrs.) determinations repeated often enough to cover the first 30 hours of fasting. This latter procedure, as already explained, involved introduction of age, weight and sea-

sonal variables which probably are enough to account for the lack of smoothness of the final average curves so derived; for all this, however, these results are valuable as showing that none of the characteristics of the data of table 1 and figure 1 are due to any untoward effect from confinement of the animals for long periods in the metabolism chamber with the ensuing restriction of activity and water intake.

Oxygen consumption and metabolic rate. Table 1 gives the values of O_2 consumption in cubic centimeters per minute and also as percentage of the immediate, post-prandial value. Also recorded is the metabolic rate as calories per square meter per 24 hours, derived from O_2 consumption and R.Q. in the usual way, using Diack's formula (1930; S = 7.47 · Wt.) for surface area; this is
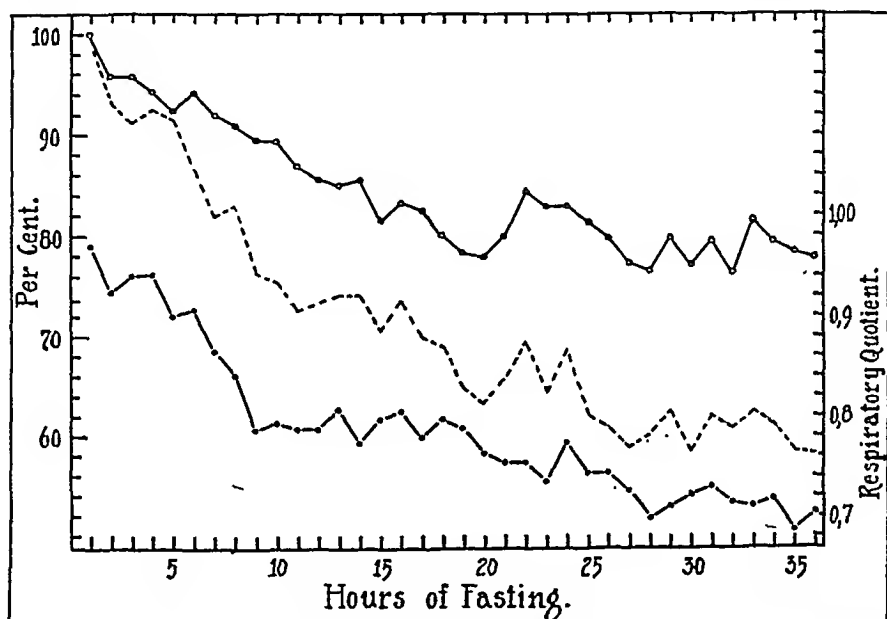
Fig. 1. The respiratory metabolism of the fasting rat for 36 hours following the ingestion of food:

Top curve (open circles): Calories per square meter. Middle curve (dashes): *Carbon dioxide output, cubic centimeter per minute.* Lowest curve (solid circles): Respiratory quotient. Calories per square meter and CO_2 production are both plotted as percentages of the maximal, immediate post-ingestion values.

also recorded as percentage of the immediate post-ingestion value, and is so used for the graph of figure 1. Oxygen consumption and calories per square meter are in no essential respect different; the latter has been chosen for illustration and may be made the basis of discussion because of its more general, comparative value.

Examination of the data shows maximal heat production of 954 Cal./m²/24 hrs. during the first hour after cessation of food ingestion. From this time until the 20th fasting hour there is decline which is continuous and uniform, to a value 78 per cent of the maximal. Such slight irregularities as there are on this part of the curve probably represent unavoidable experimental error and may be so dismissed.

Beginning at the 20th hour is an unmistakable elevation of metabolic rate, rising fairly sharply to a maximum (84.4 per cent) at the 22nd hour and returning to or slightly below the value from which it started by the 28th hour. From this time until the 36th hour there is no further major change and this latter part of the curve is perhaps best described as an oscillation about the value, 78 per cent of maximum, reached at the 20th hour.

Carbon dioxide production, also, is maximum during the first hour of fasting and thereafter declines, as already observed for O_2 consumption and heat production, but with these differences: the decline is more rapid until the 11th hour; a more or less stable period, at about 73 per cent of maximum, then supervenes until the 16th hour; minimal, stable rate of production is very clearly not reached until the 26th–27th hours, oscillating, thereafter, about a value approximately only 60 per cent of the maximum post-ingestion rate. The temporary elevation of metabolic rate observed for O_2 consumption and heat production between the 20th and 28th hours is also evident on the CO_2 curve, but less clearly, due to its super-position on a still-declining base line. The final, steady, rate of fasting CO_2 production, approximately 60 per cent of maximum, from the 28th hour on, is 17± per cent lower than the corresponding final metabolic rate as derived from O_2 consumption.

Respiratory quotient. As mentioned under Procedure, respiratory quotient showed the same trend, with no difference in hour-to-hour variability, whether derived from the short-period or continuous determinations. Age, weight and seasonal disturbances introduced by the successively-repeated short-period method, apparently had little effect on this qualitative measure, and a slightly smoother curve is obtained by averaging all observations. These, as already described, include for the first 30 hours of fasting, 10 determinations per hour by the short-period method. An equal number from continuous runs for the first 24 hours make a total of 20 determinations per hour for this period. From the 24th to the 36th hours there were also 8 continuous runs, which, with the ten short-period determinations covering the 24–30 hour period, make a total of 18 per hour for this interval, and 8 per hour for the 30–36 hour terminal portion of the curve.

As may be seen from the table and especially from figure 1, respiratory quotient declines from a maximum of 0.977 during the first fasting hour to approximately 0.79 at the 9th hour. For the next 10 hours, i.e., until the 19th, there is no further decline, but merely oscillation about this value, the actual average for the 9th to 19th hours, inclusive, being 0.793. Beginning with the 20th hour the decline is resumed, but until the 27th–29th hour is apparently somewhat influenced by the metabolic events responsible for the elevation of rate during this interval; so that the actual 24-hour value of which so much has been made is, we believe, more than just accidentally high (0.774). Between the 27th and 28th hours, however, respiratory quotient appears to have reached a minimal steady value, the average for the 28th to 36th hours, inclusive, being 0.710.

DISCUSSION. Perhaps the most obvious and necessary comment on the data now available regarding the fasting respiratory metabolism of the rat is to emphasize the urgent need for further information. That which is at hand is still

too meagre and, particularly, too completely lacking in agreement to permit any general conclusion. This is still true even after the elimination, which may be made at once and until some confirmatory evidence in their support is forthcoming, of the qualitatively bizarre results of Werthessen (1937). Even thus restricted, the remaining data are more remarkable for their diversity than for their agreement.

As far as metabolic rate is concerned, the results described here are intermediate between those reported by Wesson (1931) and by Benedict and MacLeod (1928, p. 363). The latter made observations at only occasional intervals, 17, 24, 42 and 64 hours, during fasting, obtaining average values 95, 87, 95 and 92 per cent, respectively, of the immediate post-ingestion maximum of 1065 Cal. $m^2/24$ hours (using Rubner's constant of $9.1 \cdot W^{\frac{2}{3}}$ for calculation of surface area). These values are higher than ours, absolutely, and even more so when account is taken of the use by them of 9.1 instead of 7.47 (as by us; Diack's formula) as the constant for estimation of body surface; but, more particularly, they indicate considerably less decrease in metabolic rate during fasting, than is shown by our rats for the first 24 hours. Also the conclusion from these results and others which they review, that "ingestion of food does not exert any appreciable influence on the rat's metabolism after 14 to 17 hours" (Horst, Mendel and Benedict, 1934, p. 281) is obviously far from justified by our data.

As mentioned in the introduction, Wesson is the only one, in so far as we know, previously attempting to define the fasting metabolic rate accurately hour by hour; and with results which agree with ours even less than those of Benedict and MacLeod. From a post-ingestion maximum of 1072 Cal./m^2/24 hours (which, absolutely, is even still higher than ours because of the use of Lee's constant of 12.54 in calculation of body surface), Wesson observed an abrupt, precipitous drop to 68 per cent of this value at the 4th fasting hour, and an extreme fall to only 54 per cent at the 22nd hour. In all other respects, also, his data resemble ours so little that further detailed comparison would be fruitless; even the fact that his rats received pure dextrin rather than the usual mixture of foodstuffs for their last meal hardly seems sufficient to explain the difference between his results and our own.

And, just as there is no similarity, in general, between our results and those of others, so, also, there is nothing in previous evidence by which to judge the possible correctness or significance of the decided calorigenic response evident on our curve between the 20th and 28th hours. Judged by internal evidence, it would seem not to be an artefact. It will be recalled that the total calorimetric data shown in the curve of figure 1 are composed of two separately determined sets: 1–24 hour runs and 25–36 hour runs, done, in part, on different animals and, when on the same animals, separated by intervals of about one month. There would be some reason, therefore, to expect the possibility of a break in the curve at the 24–25 hour interval where the two sets of data are joined, unless the determinations are really accurate measurements of the metabolic rate. Since, as can be seen, the continuity of the curve at this point is not broken although the junction is made at a time of rapidly changing rate, it would seem reasonable

to conclude that the data, as given, represent with reasonable accuracy the phenomenon being measured.

As to the meaning of such an effect at this time it may be significant that its onset coincides with reduction of liver glycogen to minimal values (Long et al., 1940) and, presumably, when shift is being made to other sources of energy; it, therefore, might be taken to represent an endogenous specific dynamic action, particularly of fat; an interpretation in line with the rapid reduction in respiratory quotient to a fat level occurring during the same time, to be referred to in what follows.

Previous evidence regarding the respiratory quotient of the fasting rat need not be set forth here in detail. This, which is concerned chiefly (except that of Wesson) with fixing the value characteristic of the 24-hour fasting rat, or the earliest time at which a stable, minimal value is attained, has been reviewed by Horst, Mendel and Benedict (1930, p. 178). Their conclusion is that most of the previously published values are too high; and, with a finality which has conditioned all later thinking, state that "the respiratory quotient of the rat after 24 hours of fasting is so near 0.72, on the average, that one may with confidence measure the CO_2 production or the O_2 consumption only, assume a respiratory quotient of 0.72, and compute the total metabolism with a minimum error." Later (1934, p. 281) they summarize their experience as indicating "respiratory quotients not far from 0.72 in 16 to 23 hours after food;" a deduction clearly at such variance with our results that comment or attempt at reconciliation is completely stopped.

There is no suggestion in any of this previous evidence regarding respiratory quotient of the complicated pattern of change indicated by our results; and, yet, this seems metabolically reasonable. It would appear to be demanded that at some stage in the post-absorptive metabolism this should stabilize, qualitatively, with combustion of the average mixture of foodstuffs and an R.Q. in the neighborhood of 0.80. This, our results show to be true between the 9th and 19th hours. By the end of this time, as is now well known for the rat, liver glycogen is reaching minimal values and as our results show, shift to other than carbohydrate sources of energy is accompanied by a definite effect on total heat production and is reflected in an abrupt decline of respiratory quotient. Only by the time this alteration is apparently complete, i.e., the 28th fasting hour, does the R.Q. reach a true fat value, which is then maintained for as long as our data go, to the 36th hour.

In concluding it may be mentioned that, if our results are correct, it is unfortunate the 24-hour fasting rat has been chosen, somewhat blindly and empirically, as a standard animal for metabolic work; for this apparently is a time of particular metabolic instability. This might not be serious if determinations were always made at exactly this time by employment of trained, controlled feeding; but as this is almost never done the prescription is more honored in the breach than the observance. Thus Horst, Mendel and Benedict, with a candor which is unique, acknowledge (1934, p. 281) the probability that actual fasting time may easily be 3 to 4 hours longer than its usual, uncritical estimation.

This would mean that the usual (inadequate) precautions taken to ensure a 24-hour fast result in determinations made 27–28 hours after actual ingestion of food and thus at a time when the metabolism, according to our evidence, is actually becoming minimal and steady both as to rate and respiratory quotient; and thus providing ground for those who insist that both of these are true for the alleged 24-hour period.

Our results do not provide any equally plausible alibi for those who have placed the attainment of minimal metabolic rate and respiratory quotient as early as the 14th–17th hours. But if, on the assumption that any times beyond this and approximating 24 hours were safely minimal and classifiable for rough identification as a 24-hour fast, determinations are made on animals presumably fasted for 20–24 hours, the data would no doubt really belong to the 22–26 hour interval; for which, according to our results the average R.Q. is 0.75; a figure exactly equal to that reported by Schwabe et al. (1938) as the average of some 400 determinations so made. From all of which it follows that in future such rough approximations of fasting time will have to be candidly admitted or greater pains used to secure more accurate timing.

SUMMARY

The fasting respiratory metabolism of the albino rat (average age 11 months; weight, 340 grams) is described hour-by-hour for the first 36 hours following controlled feeding.

Metabolic rate calculated from O_2 consumption and R.Q. in the usual way and using Diack's formula for estimation of body surface, drops from an immediate post-ingestion maximum of 954 Cal./m²/24 hours steadily and uniformly to 78 per cent of this value at the 20th fasting hour. During the next 8 hours the curve is elevated in semblance of a specific dynamic reaction to fat, with maximum of 84 per cent at the 22nd hour and final return to 76.6 per cent by the 28th hour. Thereafter, until 36 hours, the general trend is horizontal with oscillation about the value, 78 per cent, previously reached at the 20th hour.

Respiratory quotient drops from an immediate post-ingestion maximum of 0.970 to approximately 0.790 at the 9th hour. Thereafter, until the 19th hour it indicates the combustion of the usual, post-absorptive mixture of foodstuffs and merely oscillates about this as an average value. Beginning with the 20th hour decline is resumed to reach 0.70 at the 28th hour, but is interrupted by a temporary rise between the 22nd and 26th, to a maximum of 0.774 at the 24th hour. Beyond the 28th and until the 36th hour there is mere oscillation about an average fat value of 0.710.

These results, if true and confirmed, indicate the 24-hour fasting rat was a peculiarly unfortunate choice for a metabolic standard, since this point is apparently midway in the transition from the metabolism of mixed foodstuffs to one exclusively of fat; consequently the slightest inaccuracy in estimation of fasting time at this particular interval will necessarily eventuate in results of relatively wide diversity.

REFERENCES

BENEDICT, F. G.   J. Nutrition 3: 160, 1930.

BENEDICT, F. G. AND G. MACLEOD.   J. Nutrition 1: 343, 1929a.
          J. Nutrition 1: 367, 1929b.

BUNNELL, I. AND F. R. GRIFFITH, JR.   Proc. Soc. Exper. Biol. and Med. 44: 509, 1940.

DIACK, S. L.   J. Nutrition 3: 289, 1930.

HORST, K., L. B. MENDEL AND F. G. BENEDICT.   J. Nutrition 3: 177, 1930.
          J. Nutrition 7: 277, 1934.

KLEIBER, M. AND A. H. SMITH.   Proc. Soc. Exper. Biol. and Med. 45: 630, 1940.

LONG, C. N. H., B. KATZIN AND E. G. FRY.   Endocrinology 26: 309, 1940.

SCHWABE, E. L. AND F. R. GRIFFITH, JR.   J. Nutrition 15: 187, 1938.

SCHWABE, E. L., F. E. EMERY AND F. R. GRIFFITH, JR.   J. Nutrition 15: 199, 1938.

WERTHESSEN, N.   This Journal 120: 458, 1937.

WESSON, L. G.   J. Nutrition 3: 503, 1931.

THE RÔLE OF THE ANTERIOR PITUITARY IN ADRENALINE HYPERGLYCEMIA AND LIVER GLYCOGENOLYSIS[1,2]

R. C. DE BODO, H. I. BLOCH AND I. H. GROSS

From the Department of Pharmacology, New York University College of Medicine

Convincing evidence is available that the anterior pituitary influences the breakdown of endogenous protein and the mobilization of depot fat, either directly or through the mediation of other endocrine glands. Thus, it has been shown that the hypophysectomized animal during fasting has a decreased nitrogen output (Aschner, 1912) and a diminished gluconeogenesis from protein (Houssay, 1936); and that the migration of fat from the fat depots to the liver as caused in normal animals by carbon tetrachloride or phosphorus (Issekutz and Verzár, 1938), or by pancreatectomy (Long and Lukens, 1936), is prevented by hypophysectomy. Furthermore, thyroid feeding cannot deplete the stored depot fat in hypophysectomized as it does in normal animals (Cope and Marks, 1934–1935).

In view of this established influence of the anterior pituitary on the mobilization of protein and fat, the question arises as to its rôle in the mobilization of carbohydrate. Experiments carried out on hypophysectomized animals in which the effects of adrenaline on blood sugar and glycosuria were studied might have thrown some light on the question. However, the literature on this subject is contradictory.

Aschner (1912) was the first to show that adrenaline produces either slight glycosuria or none at all in hypophysectomized dogs. Corkill, Marks and White (1934) demonstrated that adrenaline administered subcutaneously to hypophysectomized rabbits produced less hyperglycemia than in normals, despite previous glucose feeding. Similar findings have been reported by many investigators (Cope and Marks, 1934–1935; Houssay and di Benedetto, 1932; Chaikoff et al., 1935; Bachman and Toby, 1936; Collip et al., 1937).

Russell and Cori (1937) criticized this work on the grounds that the subcutaneously administered adrenaline was not absorbed by the hypophysectomized animals. When they administered adrenaline intravenously they found the hyperglycemic response in their hypophysectomized rats was the same as in their normals. This contradicts the work of Braier (1931), who also administered adrenaline intravenously but found that hypophysectomized dogs responded with far less hyperglycemia than normals. It also contradicts the results of Fluch, Greiner and Loewi (1935) and of Képinov (1937a, 1937b), who found diminished glycogenolysis in the isolated livers of hypophysectomized frogs when they added adrenaline to the perfusing fluid.

124

The experiments of Russell and Cori (1937) are open to criticism. They presented results obtained on four hypophysectomized rats in which, after the intravenous infusion of adrenaline, they observed changes in blood sugar of +40, +133, +47, and +93 mgm. per cent. The average of these widely divergent figures was compared with the average of similarly divergent figures obtained in five normal rats. Their conclusion based on the comparison of these averages is not justified. Furthermore their animals were not in the postabsorptive state—"were not fasted prior to the experiment"—and therefore might have been absorbing varying amounts of carbohydrate from their gastro-intestinal tracts. Finally, even had their experiments been otherwise satisfactory, since they claim to have found no difference in response to intravenously administered adrenaline between their normal and their hypophysectomized animals, it is essential that they produce conclusive histological evidence that their animals were completely hypophysectomized. They failed to do so. Considering all these facts their results are not convincing.

Heinbecker and Weichselbaum (1937) administered adrenaline intraperitoneally and found that the hyperglycemic response was the same in their hypophysectomized as in their normal animals. They state that their hypophysectomized dogs were "2–4 times as sensitive [to insulin] as the normals." An analysis of their tables reveals that the post-absorptive blood sugars of their hypophysectomized dogs hardly differed from those of their normals, the former ranging from 57 to 79 mgm. per cent (average 67 mgm. per cent) and the latter from 60 to 86 mgm. per cent (average 72 mgm. per cent). This is in contrast to our own findings. Our hypophysectomized dogs were 30–60 times as sensitive to insulin as normals. The post-absorptive blood sugars of our hypophysectomized dogs were considerably lower than those of normals (see figures given below). Consideration of these findings and the additional fact that the completeness of their hypophysectomy was not verified by histological study (which is again indispensable in view of their findings) makes it doubtful that the animals which Heinbecker and Weichselbaum used for their experiments were completely hypophysectomized.

Since exact knowledge as to the hyperglycemic and glycogenolytic action of adrenaline in the hypophysectomized animal would help to clarify the rôle of the anterior pituitary in carbohydrate metabolism, and since the literature on this subject contains many contradictions, we reinvestigated this question.

METHODS. Our experiments were done on dogs—24 normal, 13 hypophysectomized, and 6 neurohypophysectomized.

In the earlier series the hypophysectomies were performed by the temporal approach, in the later series the oral approach was used. In both, the entire hypophysis (pars distalis, pars nervosa, pars intermedia, and pars tuberalis) was removed in one piece. When the oral approach was used the bone opening was plugged with a sulfathiazole tablet (0.5 G.), and the animals were given 1 gram of sulfathiazole by mouth daily (divided into two doses) following the operation, until the stitches were removed from the soft palate. No postoperative infection occurred in any of our hypophysectomized animals. All the animals

discussed herein took food voluntarily within 24 hours after the operation. Following the operation the post-absorptive blood sugar levels were determined almost daily. Blood sugar determinations were also made after varying periods of fasting. Subsequently the animals were tested for their sensitivity to insulin. These studies constituted a valuable guide to the degree of completeness of our hypophysectomy before the animals were sacrificed. Later the completeness of the hypophysectomy was actually determined by histological study of sections of a block including the body of the sphenoid bone, the fibrous tissue occupying the sella turcica, and the overlying brain tissue. In addition sections were made of the organs, with special attention to the thyroids, adrenals and gonads, and also of the removed pituitary gland. These studies were made by Dr. David Marine, Director of the Laboratories of Montefiore Hospital, New York City.

The neurohypophysectomies were performed by the temporal route. The entire neurohypophysis [infundibular process (neural lobe), infundibular stem, and median eminence] was destroyed, leaving the anterior lobe intact. These animals had permanent diabetes insipidus—excreting an average of 4000 to 5000 cc. of urine per day, during the two years following the operation.

All the animals (normal, hypophysectomized and neurohypophysectomized) were maintained on a constant diet. All the experiments were done with the animals in the post-absorptive state, seventeen to eighteen hours after the last feeding. In every case it was ascertained that the animal consumed and retained the allotted diet. However in one group of normal animals the experiments were made after an 8 to 13 day fast.

The experiments were made without the use of any anesthetic. The dogs were trained to lie quietly during the procedures. Adrenaline was infused intravenously, at a constant rate of 0.0035 mgm./kgm./min. for five minutes. This rate of infusion is within the limits of the physiological adrenaline output in cats, as determined by Cannon and Rapport (1921). We consider theirs the only valid method of determining adrenaline output in animals under physiological conditions. To our knowledge no figures determined by their method are available in the literature for the physiological adrenaline output of dogs. However, this amount of adrenaline was found to produce a marked hyperglycemia in all our normal dogs.

Blood sugar samples were taken before the adrenaline infusion was begun, at the end of the infusion, and at regular intervals thereafter for the next two and one-half hours. The adrenaline experiments were repeated on some of the animals, at intervals of several days. Blood sugar was determined by the Hagedorn-Jensen method (1923), using the Somogyi acid-zinc filtrate (1930).

Liver samples were excised under local anesthesia for glycogen determination. They were excised a few days after the adrenaline experiments (method 1—indirect), blood being drawn for sugar determination prior to the excision. During the interval between the adrenaline experiment and the excision of liver samples, diet being constant, post-absorptive blood sugars and body weights were followed daily. In some animals, after the liver samples were excised, blood was again drawn for sugar determination, and the adrenaline infusion experiment

was repeated.   Additional liver samples were taken fifteen minutes after the end of the infusion (method II—indirect plus direct).   Liver glycogen was determined by a modified Pflüger method (Bodo and Neuwirth, 1933).

RESULTS.   *Effect of adrenaline on blood sugar of normal dogs.*   The five-minute intravenous infusion of adrenaline (0.0035 mgm./kgm./min.) into eighteen normal, unanesthetized dogs produced a marked hyperglycemic response.   The post-absorptive blood sugar values of these animals ranged from 79 to 93 mgm. per cent (average 85 mgm. per cent).   The day-to-day variation in post-absorptive blood sugar value of each of these dogs was found to be less than 10 mgm. per cent during three weeks of observation.   The rise of blood sugar caused by adrenaline was apparent by the end of the infusion, and reached its maximum within fifteen minutes from the start of the infusion.   The average maximum rise of blood sugar was 58 mgm. per cent.   The smallest rise seen in a normal animal was 43 mgm. per cent and the greatest rise was 81 mgm. per cent.   The blood sugar gradually returned to normal levels within 120 minutes from the start of the infusion.

Effect of adrenaline on blood sugar of hypophysectomized dogs.   The blood sugar changes induced in thirteen unanesthetized hypophysectomized dogs by the five-minute intravenous infusion of adrenaline (0.0035 mgm./kgm./min.) are recorded in table 1.   Of these thirteen dogs, in dogs H7, H9, H10, H12 and H13 histological study of sections of a block including the body of the sphenoid bone, the fibrous tissue occupying the sella turcica, and the overlying brain tissue definitely established the complete absence of pituitary cells.   The pars distalis, pars nervosa, pars intermedia, and pars tuberalis had all been completely removed.   In dog H11 a few strands of blurred cells were found, which might have been pituitary cells.   In all the above dogs the hypophysectomies were done by the oral approach.   In each the adrenal cortices, thyroids, and gonads showed the atrophic changes characteristic of complete hypophysectomy, except that some slight evidence of activity was found in the gonads of dog H11.   The remaining dogs listed in table 1 were hypophysectomized by the temporal approach.   All were found to have small remnants of anterior pituitary tissue. However, these remnants were functionally insufficient to prevent the atrophy of the adrenal cortices, thyroids, and gonads, and they were not sufficient to influence metabolism.   These dogs were found to be as sensitive to insulin as the 100 per cent completely hypophysectomized dogs were.   All these animals were 30 to 60 times as sensitive to insulin as the normal dogs.   Furthermore, these sub-totally hypophysectomized dogs reacted exactly as did the totally hypophysectomized dogs to varying periods of starvation, and showed similar low post-absorptive blood sugar levels and the same tendency to spontaneous hypoglycemic crises.   On this basis we have considered the sub-totally hypophysectomized dogs as functionally hypophysectomized.   The effect of adrenaline on the blood sugar levels of both totally and sub-totally hypophysectomized dogs are considered in table 1.

As may be seen, the post-absorptive blood sugar values of the hypophysectomized dogs varied from 33 to 69 mgm. per cent (average 53 mgm. per cent)—

TABLE 1

The effect of intravenously infused adrenaline on blood sugar of hypophysectomized dogs. Liver glycogen determination by method 1

Rate of intravenous adrenaline infusion: 0.0035 mgm./kgm./min. for five minutes. All experiments started 18 hours after last feeding. No anesthetic given.

DOG NUMBER	WEIGHT AT OPERATION	DAYS AFTER HYPOPHYSECTOMY	WEIGHT AT EXPERIMENT	BLOOD SUGAR (MGM. PER CENT)			LIVER GLYCOGEN
				Before adrenaline*	Maximum after adrenaline	Increase	
	kgm.		kgm.				per cent
H 5	14.6	43	15.0	55	68	13	
		52	14.6	58	69	11	
		60	14.9	54			4.42
H 6	14.8	49	15.1	56	71	15	
		58	15.2	54			4.02
H 7	13.0	7	12.1	49	76	27†	
		24	13.0	43	50	7	
		27	12.9	52			3.95
H 8	15.8	42	15.6	56	69	13	
		53	15.8	53			2.86
H 9	17.0	26	17.6	67	84	17	
		33	18.0	56	71	15	
		36	17.6	59			2.05
H 10	15.9	22	16.9	69	88	19	
		26	16.6	51			1.65
H 11	23.8	14	22.8	41	51	10	
		18	21.6	38			1.22
H 12	13.9	24	14.8	52	67	15	
		31	14.8	33			0.94
H 13	14.3	23	14.1	46	56	10	
		27	13.1	33			0.98
H 1	18.5	40	19.0	62	77	15	‡
H 2	19.0	37	18.6	69	82	13	‡
H 3	15.4	34	15.5	61	74	13	‡
H 4	14.4	34	15.0	59	66	7	‡
Average...						13	

* Postabsorptive value.
† Not included in average.
‡ Liver glycogen not determined.

far below that of the normals (average 85 mgm. per cent). The wide range of these values seen in the hypophysectomized dogs is related to the progression of the changes following the hypophysectomy. This relation will be discussed at length in a subsequent paper.

As in the normal dogs, the hyperglycemic response in the hypophysectomized dogs is apparent by the end of the adrenaline infusion, and reaches its maximum values within fifteen minutes from the start of the infusion. However, the blood sugar returns to its post-absorptive level more promptly than it does in the normal animals. As will be noted in table 1, the intravenously infused adrenaline produced a maximum rise in blood sugar of 7 to 19 mgm. per cent (average 13 mgm. per cent) in the hypophysectomized dogs—far less than that produced in the normal dogs (average 58 mgm. per cent). We have not included the rise of 27 mgm. per cent observed in dog H7 in an experiment carried out only seven days after hypophysectomy. In a second experiment carried out on the same dog twenty-four days after hypophysectomy a maximum rise of only 7 mgm. per cent occurred (see table 1), and in a third experiment carried out twenty-seven days after hypophysectomy the maximum rise was 10 mgm. per cent (see table 3). In all the other experiments on all the other animals, an interval much longer than seven days had elapsed between the hypophysectomy and the adrenaline experiment, and invariably only a slight hyperglycemic effect was observed. This would suggest that the reduced hyperglycemic response to adrenaline is not present immediately after hypophysectomy but develops only subsequently. In connection with the rise of 27 mgm. per cent observed in dog H7 seven days after hypophysectomy it is also significant that whenever the adrenaline experiment was repeated on any of the other dogs (see dogs H5, H9 in table 1, and dogs H9, H11 and H12 in tables 1 and 3) adrenaline produced almost identical increases in blood sugar on each occasion. Even dog H7 gave two consistent figures on repeated experiments (see tables 1 and 3). For these reasons we have considered the isolated figure of 27 mgm. per cent separately.

Liver glycogen content of hypophysectomized dogs—Determined by method 1. Having found a diminished hyperglycemic response to adrenaline in hypophysectomized dogs the possibility suggested itself that this might have been due to the absence of adequate liver glycogen stores. Therefore it became essential to determine the amount of liver glycogen available in these dogs for mobilization by the infused adrenaline. Excision of liver samples before the infusion of adrenaline was deemed inadvisable due to its possible effect on the blood sugar curve; and it is obvious that a determination made at the end of an adrenaline experiment would give no indication of the amount of liver glycogen available at the beginning of that experiment. Therefore the procedure finally adopted was, as described above, to excise liver samples a few days after the last of the adrenaline experiments, while the animals were maintained on a constant diet. In our opinion this gives an acceptable index of the liver glycogen present at the time of the infusion, if the body weight and the post-absorptive blood sugar level remained practically constant during the interval between the adrenaline experiment and the excision of liver samples.

In the experiments on dogs H5, H6, H7, H8, and H9 (table 1) these conditions were fulfilled, and we believe that the glycogen values obtained are very close to those actually present at the time of the adrenaline experiment. In the experiments on dogs H10, H12 and H13 the post-absorptive blood sugar values at the time of the adrenaline experiment were much higher than they were at the time the liver samples were taken. Therefore it is most probable that the liver glycogen values were also much higher at the time of the adrenaline experiments than they were when determined. In dog H11 there was a decrease in body weight, and therefore the liver glycogen as determined is probably lower than that present at the time of the adrenaline infusion. Dogs H1, H2, H3 and H4 constituted an earlier series and no liver glycogen determinations were made on them, but they had relatively high post-absorptive blood sugar values and, in consideration of the glycogen values found in dogs H10, H11, H12 and H13 coexisting with much lower post-absorptive blood sugar values, it is probable that they too had ample liver glycogen stores.

Even disregarding the probability, that some of these animals had higher liver glycogen values at the time of the adrenaline experiment than they had at the time the determinations were made, it is obvious that at least H5, H6, H7 and H8 had liver glycogen values within the range of those found in normal dogs. In a series of normal dogs in the post-absorptive state we found glycogen values ranging from 2.9 to 7.0 per cent. As for the others, dogs H9, H10, H11, H12 and H13 had amounts of liver glycogen which were adequate to produce a hyperglycemia far greater than that observed, had their liver glycogen been available. This point is clearly brought out by a study of the hyperglycemic response to adrenaline infusion in a series of fasted normal animals.

Effect of adrenaline on blood sugar of fasted normal dogs. The blood sugar rises produced by the intravenous infusion of adrenaline (0.0035 mgm./kgm./min.) in a series of normal dogs fasted for periods varying from 8 to 13 days are shown in table 2. In dogs F1, F2, F3 and F4 the experiment was repeated. As in the normal and the hypophysectomized dogs, the blood sugar rise is apparent by the end of the infusion and reaches its maximum within fifteen minutes from the start of the infusion. However, the blood sugar did not return to its pre-adrenaline level during the course of two and one-half hours. The maximum rise in blood sugar ranged from 27 to 37 mgm. per cent (average 31 mgm. per cent). The liver glycogen values of the fasted normal animals ranged from 0.7 to 1.3 per cent (average 1.0 per cent).

The average rise of 31 mgm. per cent in the blood sugar values of these normal animals fasted 8 to 13 days is more than twice that observed in the hypophysectomized series in the post-absorptive state (13 mgm. per cent) despite the fact that the liver glycogen stores of the fasted normals were no higher than the lowest values found in our hypophysectomized dogs. In other words the lowest of the liver glycogen values found in our hypophysectomized dogs was ample to have produced twice the observed hyperglycemia.

Liver glycogen content of hypophysectomized dogs—Determined by method 2. As stated above, in all the hypophysectomized animals, liver samples were taken a

few days after the adrenaline experiment (method 1—indirect). However, in dogs H7, H9, H11 and H12, following this excision of liver samples another adren-

TABLE 2

The effect of intravenously infused adrenaline on blood sugar of fasted normal dogs

Rate of intravenous adrenaline infusion: 0.0035 mgm./kgm./min. for five minutes. No anesthetic given.

DOG NUMBER	PERIOD OF FASTING	BLOOD SUGAR (MGM. PER CENT)		
		Before adrenaline	Maximum after adrenaline	Increase
	days			
F 1	8	67	94	27
	10	58	94	36
F 2	8	59	87	28
	10	58	93	35
F 3	8	63	92	29
	10	66	93	27
F 4	8	76	113	37
	13	76	112	36
F 5	10	73	105	32
F 6	8	59	86	27
Average..				31

TABLE 3

The effect of intravenously infused adrenaline on blood sugar and liver glycogen of hypophysectomized dogs. Liver glycogen determination by method 2

Rate of intravenous adrenaline infusion: 0.0035 mgm./kgm./min. for five minutes. All experiments started 18 hours after last feeding. No general anesthetic given.

DOG NUMBER	WEIGHT AT OPERATION	DAYS AFTER HYPOPHYSECTOMY	WEIGHT AT EXPERIMENT	BLOOD SUGAR (MGM. PER CENT)				LIVER GLYCOGEN	
				Before adrenaline		Maximum after adrenaline	Increase	Before adrenaline	After adrenaline
				Before liver sample*	After liver sample				
	kgm.		*kgm.*					*per cent*	*per cent*
H 7	13.0	27	12.9	52	52	62	10	3.95	3.67
H 9	17.0	36	17.6	59	72	88	16	2.05	1.40
H 11	23.8	18	21.6	38	40	47	7	1.22	0.84
H 12	13.9	31	14.8	33	32	42	10	0.94	0.79

* Postabsorptive value.

aline infusion was started. Fifteen minutes from the end of this infusion additional liver samples were excised (method II—indirect plus direct). The results

of these experiments are shown in table 3. It will be noted that dog H7 which had 3.95 per cent liver glycogen at the time of the adrenaline infusion showed a maximum blood sugar rise of only 10 mgm. per cent. Similarly, dogs H9, H11 and H12 had 2.05, 1.22 and 0.94 per cent liver glycogen and showed maximum blood sugar rises of 16, 7 and 10 mgm. per cent, respectively. Further it should be noted that, except in dog H9, excision of the first liver sample (before adrenaline) caused no rise in blood sugar. In dog H9 the blood sugar rose from 59 to 72 mgm. per cent after excision of the first liver sample. This was due to manipulation of the liver and possibly to the effect of reflexly secreted adrenaline. Obviously this obscured somewhat the effect of the subsequently infused adrenaline. None the less the total blood sugar rise calculated from the post-absorptive level is still only 29 mgm. per cent, with a liver glycogen content of 2.05 per cent.

Effect of adrenaline on blood sugar of neurohypophysectomized dogs. Up to this point we have considered the diminished hyperglycemic response to adrenaline as a characteristic of the hypophysectomized dog, but it is of interest to investigate the relative importance of the different parts of the hypophysis in the phenomenon. Geiling et al. (1927) have presented evidence that the absence of the posterior pituitary is the determining factor in insulin sensitivity, and it might therefore be expected to have a part in the adrenaline resistance which we are engaged in investigating. To elucidate the relative importance of the anterior and posterior pituitary in this phenomenon we repeated the adrenaline infusion experiments in a series of six dogs in which the anterior pituitary was left intact but the entire neurohypophysis was destroyed. Without exception these animals had normal post-absorptive blood sugar levels (93, 75, 87, 77, 75, 86 mgm. per cent), withstood fasting for a period of eight days as well as normal animals, and showed a response to insulin somewhat greater than that of normal dogs but by no means comparable to that of completely hypophysectomized dogs. When the same amount of adrenaline (0.0035 mgm./kgm./min. for 5 min.) was infused intravenously their maximum blood sugar rises in milligrams per cent were: 61, 63, 50, 71, 49, 67 (average 60 mgm. per cent). Their hyperglycemic response differed in no way from that of the normal animals. Therefore the decrease in hyperglycemic response to adrenaline is due to the absence of the anterior pituitary and is unrelated to the posterior pituitary.

DISCUSSION. On the basis of our experiments it is clear that hypophysectomized dogs invariably differ from normal dogs in that they show only a slight hyperglycemic response to intravenously administered adrenaline whereas the normals always show a marked hyperglycemia. This diminished hyperglycemic response in hypophysectomized dogs might be attributed either to a lack of adequate liver glycogen stores, or if these can be proved to have been ample, to some interference with the mobilization of the glycogen. Therefore, exact knowledge of the liver glycogen content at the beginning of the adrenaline infusion is essential.

The literature contains very few data concerning this point. Houssay and di Benedetto (1932) working with toads, and Fluch, Greiner and Loewi (1935)

working with isolated frog livers, determined the glycogen present at the time they demonstrated decreased glycogenolysis by adrenaline. Other workers who found smaller responses to adrenaline in hypophysectomized animals than in normals and attributed this to a defective glycogenolytic mechanism, either failed to do actual liver glycogen determinations, or did them under such conditions that they have no true significance. These previous workers have not done liver glycogen determinations in their adrenaline resistant animals at the time that they were adrenaline resistant. The mere fact of feeding glucose (Corkill et al., 1934) previous to an adrenaline experiment does not necessarily mean that adequate liver glycogen stores are present in hypophysectomized animals, since it is doubtful whether these animals are capable of storing glycogen even though they are absorbing sugar when there is marked atrophy of the adrenal cortex. Similarly, liver glycogen figures obtained on animals dying in insulin shock (Corkill et al., 1934) cannot be taken as any indication of the liver glycogen values present in other animals at the time of an adrenaline experiment, especially in consideration of the known glycogenopexic action of insulin.

In view of the fundamental importance of determining the liver glycogen content precisely, we adopted two methods of determination: a direct and an indirect method. In the indirect method the determination of liver glycogen was made a few days after the last adrenaline experiment, but since in the interval these animals were on a constant diet, maintained constant body weight, and showed only negligible changes in their post-absorptive blood sugar levels, it is valid to assume that their liver glycogen content in the post-absorptive state at the time of the adrenaline experiment must have been practically the same as that found in the samples excised later. In the direct method the liver samples were excised before the adrenaline was infused. The indirect method has the advantage that the blood sugar curve after the adrenaline is not exposed to the influence of opening the abdomen and taking the liver samples. The direct method has the advantage that the blood sugar and liver glycogen values are determined simultaneously; but it has the disadvantage that the blood sugar curve might be influenced by the operative procedure. As a matter of fact, with one exception it was not, as can be seen by a comparison of the figures in table 3.

Using these methods we were able to show that our hypophysectomized dogs had amounts of liver glycogen which were adequate to have produced marked hyperglycemia had they been available. Some of these dogs had normal amounts of liver glycogen, and even those with the smallest amounts were within the range of the liver glycogen found in fasted normal animals which showed a far greater hyperglycemia after adrenaline. Further evidence that the amount of liver glycogen present was not the factor limiting the hyperglycemic response is seen in the fact that regardless of the fairly wide range in liver glycogen values found in our hypophysectomized dogs their hyperglycemic response to adrenaline bears absolutely no relation to their liver glycogen values. (This fairly wide range of liver glycogen figures is related to the stage to which the changes following the hypophysectomy had progressed.)

Thus, we are forced to conclude that there is definite impairment in the mobiliza-

tion of the glycogen in response to infused adrenaline in the absence of the hypophysis. Since animals in which the entire neurohypophysis is destroyed, leaving the anterior lobe intact, respond to adrenaline exactly as normals do, this impairment of liver glycogen mobilization is due to the absence of the anterior pituitary. We believe that this conclusion is justified although some of our hypophysectomized animals had small remnants of anterior pituitary tissue. These sub-totally hypophysectomized animals behaved in every respect (response to insulin, to fasting; post-absorptive blood sugar level; tendency to spontaneous hypoglycemic crises, etc.) like the completely hypophysectomized animals, and showed the same atrophic changes in their adrenal cortices, thyroids, and gonads, and therefore may be considered functionally hypophysectomized.

The mechanism for mobilization of liver glycogen is impaired then in the absence of the anterior pituitary. However it is not completely abolished since the liver glycogen may still be mobilized under certain circumstances. It is not fixed, as can be seen from the fact that in the hypoglycemic state that precedes death in many hypophysectomized animals the liver glycogen is very low.

The liver glycogen might even have been mobilized by an amount of adrenaline larger than that which we infused. That is beside the point. It was our purpose to determine whether or not a difference could be detected between normal and hypophysectomized dogs with respect to the availability of their liver glycogen, and the dose of adrenaline which we used made a distinct difference manifest. Actually the dose selected is probably close to that which the animal might secrete under conditions of stress, and hence the results have additional interest in connection with the physiology of carbohydrate metabolism of the hypophysectomized animal.

This work reveals that hypophysectomy impairs the mobilization of liver glycogen, one of the body's chief carbohydrate stores, in response to adrenaline, one of the body's chief carbohydrate mobilizing agents. Therefore it may be concluded that the anterior pituitary—either directly or indirectly through the mediation of other endocrine glands—plays as definite a rôle in the mobilization of carbohydrate as it does in the mobilization of protein and fat.

SUMMARY

1. Adrenaline infused intravenously at the rate of 0.0035 mgm./kgm./min. for five minutes produces a marked hyperglycemia in normal dogs.

2. Adrenaline infused intravenously at the rate of 0.0035 mgm./kgm./min. for five minutes produces only a slight hyperglycemia in hypophysectomized dogs.

3. This failure of hypophysectomized dogs to respond to adrenaline with a marked hyperglycemia occurs despite the presence of ample liver glycogen stores.

4. Fasted normal dogs with much smaller liver glycogen content respond to adrenaline with a far greater hyperglycemia than the hypophysectomized.

5. Neurohypophysectomized dogs, with intact anterior pituitary, respond to the hyperglycemic action of adrenaline exactly as normal dogs do.

6. In the absence of the anterior pituitary the liver glycogen is less readily mobilized by adrenaline.

REFERENCES

ASCHNER, B. Pflüger's Arch. **146**: 1, 1912.

BACHMAN, C. AND G. TOBY. This Journal **87**: 1, 1936.

BODO, R. C. AND I. NEUWIRTH. This Journal **103**: 5, 1933.

BRAIER, B. Compt. rend. Soc. de biol. **108**: 491, 1931.

CANNON, W. B. AND D. RAPPORT. This Journal **58**: 308, 1921.

CHAIKOFF, I. L., F. L. REICHERT, L. S. READ AND M. E. MATHES. This Journal **113**: 306, 1935.

COLLIP, J. B., D. L. THOMSON AND G. TOBY. J. Physiol. **88**: 191, 1937.

COPE, O. AND H. P. MARKS. J. Physiol. **83**: 157, 1934–1935.

CORKILL, A. B., H. P. MARKS AND W. E. WHITE. J. Physiol. **80**: 193, 1934.

FLUCH, M., H. GREINER AND O. LOEWI. Arch f. exper. Path. u. Pharmakol. **177**: 167, 1935.

GEILING, E. M. K., D. CAMPBELL AND Y. ISHIKAWA. J. Pharmacol. and Exper. Therap. **31**: 247, 1927.

HAGEDORN, H. C. AND B. N. JENSEN. Biochem. Ztschr. **135**: 46, 1923.

HEINBECKER, P. AND T. E. WEICHSELBAUM. Proc. Soc. Exper. Biol. and Med. **37**: 527, 1937.

HOUSSAY, B. A. New England J. Med. **214**: 961, 971, 1936.

HOUSSAY, B. A. AND E. DI BENEDETTO. Compt. rend. Soc. de biol. **111**: 472, 1932.

ISSEKUTZ, B. VON AND F. VERZÁR. Pflüger's Arch **240**: 624, 1938.

KÉPINOV, L. Compt. rend. Acad. d. sc. **204**: 1218, 1937a.
        Compt. rend. Soc. de biol. **126**: 1084, 1937b.

LONG, C. N. H. AND F. D. W. LUKENS. This Journal **116**: 96, 1936.

RUSSELL, J. A. AND G. T. CORI. This Journal **119**: 167, 1937.

SOMOGYI, M. J. Biol. Chem. **86**: 655, 1930.

CONTINUOUSLY RECORDED ALTERATIONS IN THE BUOYANCY OF ANESTHETIZED DOGS PRODUCED BY VARIOUS RESPIRATORY MODIFIERS[1,2]

HAYDEN C. NICHOLSON AND ROBERT H. TRIMBY

From the Department of Physiology, University of Michigan, Ann Arbor

It is obvious that the volume to mass ratio of the body and consequently its buoyancy is changing throughout the respiratory cycle. Nevertheless, as far as we know, this fact has never been used as a means of recording respiration continuously. The possibility that the continuous recording of changes in buoyancy might possess some advantages over the ·more commonly used methods of recording respiration was suggested to us by a paper by J. M. Turner (1938) on respiratory variations in the weight of a man submerged in water.

The essential features of the recording system which we used are illustrated in figure 1. The dog, anesthetized with morphine and urethane, is placed on his back on a light dog board suspended in a tank measuring 70 x 60 x 40 cm. The trachea is connected through a cannula to an enclosed inspiratory-expiratory valve, from each side of which a rubber tube leads to a pipe attached to one side of the tank. These two pipes connect to a rebreathing tank provided with a Hutchinson spirometer. Arterial and venous cannulae are inserted, small blocks which may be cooled by circulating alcohol are applied to the vagus nerves, the neck incision is tightly closed by sutures, and the animal's nose and mouth are covered by a rubber membrane fastened with adhesive tape. The tank is then filled with isotonic saline to a level a few centimeters above the animal's chest.

Mounted above the tank is a short lever moving about a horizontal axis consisting of a steel rod supported on two ball bearing mountings. The dog board is suspended by four pieces of fish line from this lever close to the fulcrum. Changes in buoyancy of the animal as occur during the respiratory cycle will result in a rise or fall of the animal in the saline bath with a consequent movement of the lever about its axis. If these movements arc at all great the resulting inertia will make it impossible accurately to record the rapid changes in buoyancy associated with respiration. This difficulty is largely eliminated by a stiff flat steel spring which passes vertically through the center of the steel rod about which the lever moves. The tension provided by this spring keeps the movements of the lever and of the animal extremely small, making the system essentially an isometric one. The tension of the spring and the point of attachment of the dog board to the lever are adjustable. The very slight movement of the lever which docs occur is greatly magnified and recorded on smoked paper by a tambour system. An increase in buoyancy of the animal as occurs in

inspiration will result in a rise of the end of the lever from which the dog board is suspended, the consequent fall of the other end of the lever depressing the receiving tambour and elevating the recording tambour. In the kymograph tracing upstroke represents inspiration, downstroke expiration.

The advantage of this method of recording respiration over a spirometer tracing is that changes in the level of the tracing are due solely to changes in chest volume associated with changes in tonus of the respiratory muscles, whereas variations in the level of a spirometer tracing may result from either alterations in the tonus of the respiratory muscles or variations in oxygen consumption, and it is frequently difficult to distinguish between the two. We commonly recorded a spirometer tracing as well as our buoyancy record. This buoyancy method has the advantage over a chest band tracing that changes in total volume of the torso are recorded rather than variations in any one segment.
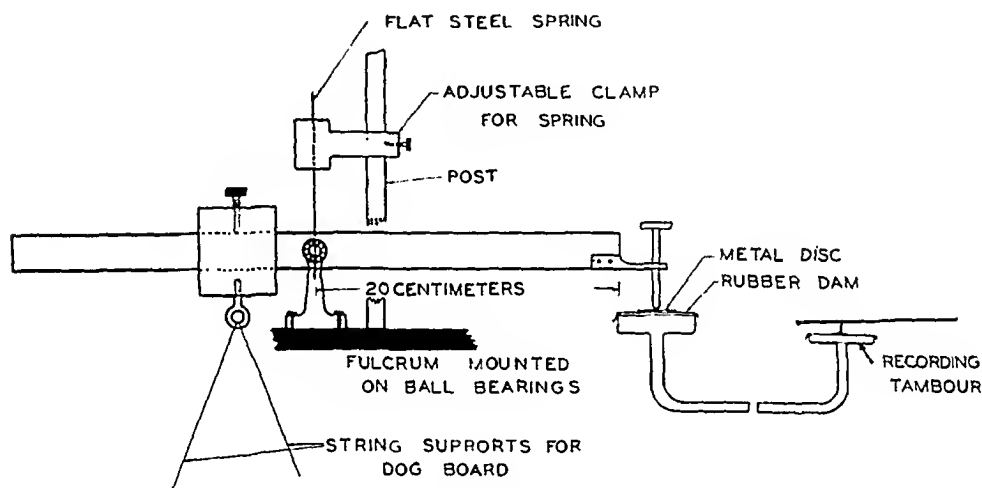
Fig. 1

The method has certain disadvantages also. If expiration is very sudden and forceful the writing point attached to the tambour tends to travel too far and may bounce one or more times. This is due in part to the flexibility of the writing point and in part to agitation of the surface of the saline in the tank and it seemed impossible entirely to eliminate it. Unless the respiratory rate is very high this is not a serious defect as the writing point has time to come to rest at its true level before the next inspiration. With very rapid breathing this cannot occur and the record may be quite inaccurate. Ordinarily, however, a record is obtained which is indistinguishable from a perfect spirometer tracing except that its level does not vary with changes in oxygen consumption.

It is true that the pressure of saline on the animal's chest may reflexly alter respiration, but this can largely be counteracted by weighting the spirometer. Ordinarily a water manometer was connected to the rebreathing tank and sufficient weight added to the spirometer to produce a pressure within the animal's lungs equal to the mean water pressure on the exterior of the chest.

The following of changes in buoyancy as a method of recording respiration

has now been applied to a number of experimental procedures commonly used in the study of various phases of respiratory physiology. Figures 2 and 3 illustrate the effects of some of these procedures upon respiration, as recorded by both the buoyancy method and the spirometer. In each case the upper record is the buoyancy record, the lower one the spirometer tracing. In some of the figures an interrupted line is drawn below the buoyancy record to make more obvious the changes in level of the tracing.
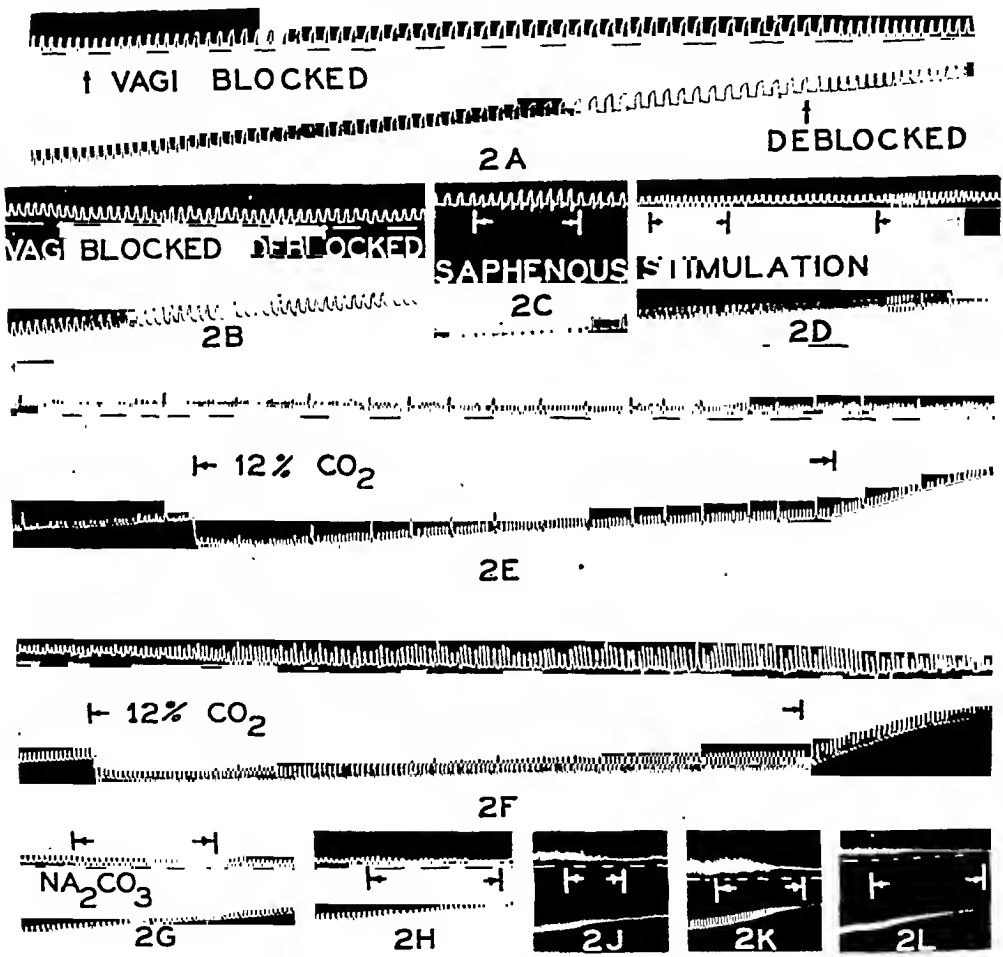
Fig. 2

Vagal block. Although blocking or cutting the vagi ordinarily increases the amplitude of breathing this occurs in spite of a decrease in the extent of expiration. That is, after vagotomy the expiratory volume of the chest is considerably greater than before, as clearly shown in figure 2A by the rise in the level of the lower edge of the tracing. Apparently the vagus normally is exerting some influence leading to a more complete expiration. This effect of vagotomy is invariably seen when, as described above, the spirometer is weighted so as to equalize the pressure on the inside and outside of the chest. If however this is not done, so that the weight of the saline in the tank exerts an unopposed pressure

tending to collapse the lungs, then the effect of vagotomy is just the reverse of
that stated above.   There now results, as illustrated in figure 2B, a more complete
expiration as shown by the drop in the level of the lower edge of the tracing.   The
inspiratory volume of the chest as represented by the upper edge of the tracing
may actually be less than normal.

These effects of vagotomy are of particular interest at the present time in
view of the concept of vagal function recently elaborated by Gesell and associates
(Worzniak and Gesell, 1939; Gesell, 1940; Gesell and Hamilton, 1941; Gesell and
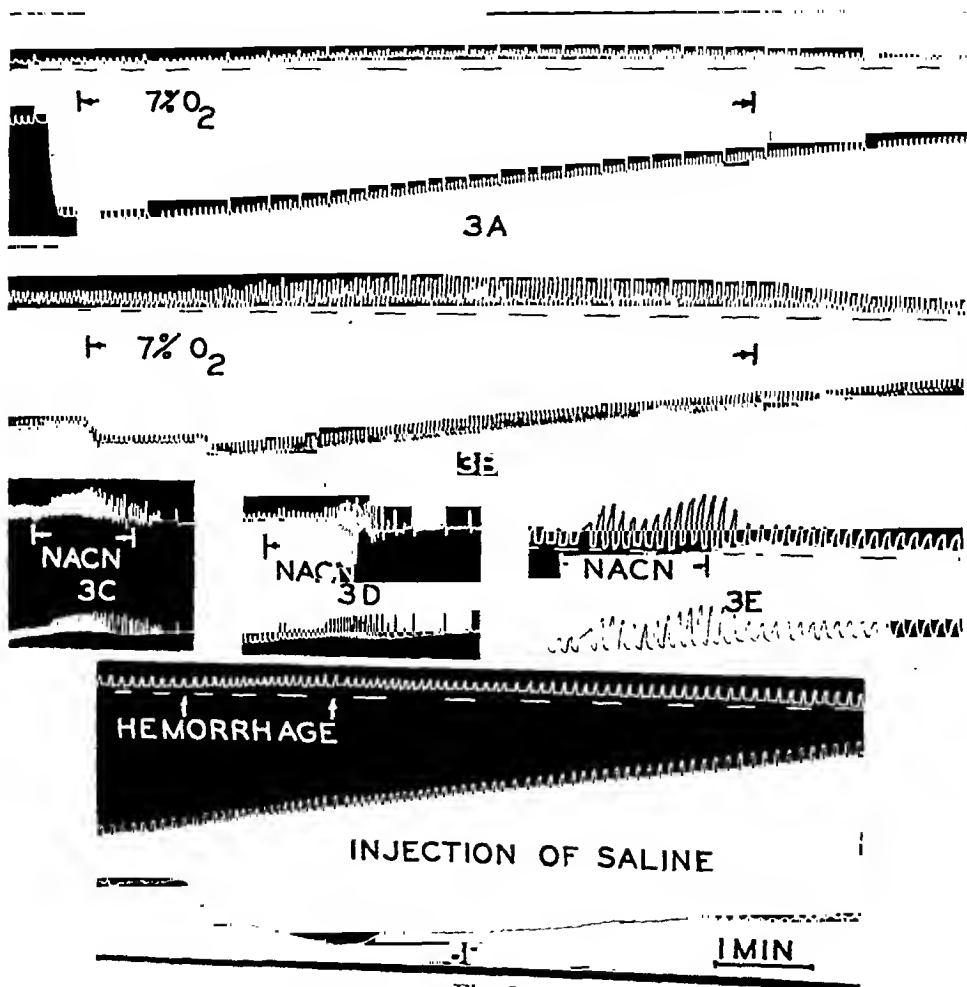
Fig. 3

Moyer, 1942), according to which vagal activity leads to a strengthening of
both inspiration and expiration, the expiratory effect ordinarily predominating.
This is in contrast to the older theories which regarded the vagus as purely in-
hibitory in function, the increase in vagal impulses with each inspiration serving
to cut short that inspiration.   The increased amplitude of respiration in the
vagotomized animal could be accounted for either by this theory or by Gesell's
view that the intact vagus excites the inspiratory half-center leading to earlier
functional exhaustion, and that the simultaneously excited expiratory half-

center by a process of reciprocal inhibition brings the inspiratory discharge to a premature conclusion. (For a more detailed discussion consult Gesell and Hamilton, 1941.) But how can the less complete expiration of the vagotomized animal illustrated in figure 2A be explained by the Hering-Breuer theory? According to this theory if in the intact animal the vagal stretch receptors alone are being stimulated vagotomy should be without effect upon the expiratory volume. If the collapse receptors also are active in bringing expiration to a premature close then vagotomy should lead to a more complete rather than a less complete expiration. The observed less complete expiration of the vagotomized animal appears capable of explanation only in terms of Gesell's concept of the vagus as an excitatory rather than an inhibitory nerve, expiratory excitation ordinarily predominating over inspiratory.

When the pressure of the saline on the outside of the animal's chest is unopposed by a similar pressure within the lungs, as was the case in figure 2B, the chest is apparently sufficiently collapsed to abolish or markedly diminish the excitation of the stretch receptors and to excite the collapse receptors. Under these conditions the vagal excitatory influence is exerted predominantly upon the inspiratory half-center leading to a high expiratory lung volume. Vagotomy now leads to a lessened lung volume as seen in figure 2B.

Saphenous nerve stimulation. In a large number of observations involving stimulation of the central end of the saphenous nerve we have never failed to observe a decrease in expiratory volume, that is, a more complete expiration. The inspiratory volume may be augmented or, if the increase in frequency of respiration is very marked, the inspiratory volume may be diminished. Three examples of such stimulation are shown in figure 2C and D. These results may be interpreted in agreement with Gesell and Hamilton (1941) as indicating a strengthening influence of such a nerve upon both inspiratory and expiratory activity.

Carbon dioxide. As is well known, the breathing of gaseous mixtures high in carbon dioxide ordinarily produces a marked increase in the amplitude of respiration. Now if one examines the buoyancy tracings in figure 2E and F it is seen that this increase in respiratory amplitude is brought about not only by a deeper inspiration but also, and to a very considerable extent by a more powerful expiration, as shown by the drop in level of the lower edge of the tracing. That carbon dioxide might markedly stimulate the expiratory half-center was indicated in the experiments of Sobin and Nicholson (1938) involving local application of carbon dioxide to the floor of the fourth ventricle and by the observation of Gesell and Moyer (1941) that pneumothorax during hypercapnia frequently caused a slowing instead of the usual acceleration of respiration, the slowing being accompanied by signs of increasing expiratory activity.

Sodium carbonate. It was found that the apnea resulting from carbonate administration may occur at or near the normal expiratory volume as seen in figure 2G, H and J or considerably above it, that is, with the chest intermediate between the inspiratory and expiratory positions as in figure 2K and L, depending apparently upon the relative dominance of inspiratory and expiratory activity.

Low oxygen. The effects of low oxygen administration contrast decidedly with those of carbon dioxide. The hyperpnea of anoxia invariably is associated with an increase in inspiratory volume. The lung volume at the end of expiration may remain constant but usually rises markedly as seen in figure 3A and B representing observations upon the same two animals as 2E and F. That is, if an increase in respiratory amplitude is to occur it must occur entirely as a result of a deeper inspiration and in spite of a less complete emptying of the lungs on expiration. This failure to increase expiratory activity may in part account for the fact that the increase in respiratory amplitude produced by low oxygen is usually considerably less than that produced by carbon dioxide administration.

Cyanide. Since the respiratory stimulating effects of cyanide are, like those of low oxygen, due mainly to action at the peripheral chemoceptors, it might be expected that the effects of the two procedures would be similar. This expectation is in large part realized if differences in the intensity of the stimulus in the two cases are taken into account. The hyperpnea of cyanide is invariably associated with an increase in inspiratory volume. The expiratory level may remain constant as in figure 3E and the early part of figure 3D, or it may rise as in the early part of figure 3C. However, there not infrequently occurs marked augmentation of expiration, something never observed upon administration of low oxygen mixtures. This effect is illustrated in the latter parts of figure 3C and D. This increased expiratory activity is most frequently seen in those observations in which the hyperpnea is maximal, it usually occurs rather late—after increased inspiratory activity has been evident for some time—and often is most evident at a time when the hyperpnea is diminishing or when signs of respiratory depression are appearing. All of these characteristics suggest that fatigue or even depression of the inspiratory mechanism following its extreme excitation may be responsible for the increased expiratory activity produced by cyanide but not by low oxygen.

The effects of anoxia and cyanidemia here described agree with the observation of Gesell and Hamilton (1941) that electrical stimulation of Hering's nerve may increase both inspiratory and expiratory activity, but that the inspiratory excitation predominates.

Hemorrhage. The hyperpnea of hemorrhage is invariably associated with a decrease in the extent of expiration, as shown by the increase in the buoyancy of the animal in figure 3F. Reinjection of blood, saline, or even distilled water reverses this effect. This result of hemorrhage *may* be due simply to the decrease in the volume of the thoracic and abdominal contents leaving more room in the chest for air.

<div align="center">SUMMARY</div>

A method of recording respiration by continuous determinations of the buoyancy of an animal submerged in water is described and its advantages and limitations discussed.

Results of the application of this method to various procedures commonly used in the study of respiration are described.

Vagotomy is followed by an increase in inspiratory volume of the lungs but a decrease in the extent of expiration, indicating that the vagus previously had been exerting a predominantly expiratory augmenting influence. If the pressure of the liquid in the tank upon the animal's chest is not compensated, thus resulting in excitation of the pulmonary collapse receptors and removal of excitation from the stretch receptors, the above effects of vagotomy are reversed, the extent of expiration now being increased, indicating that under these conditions the intact vagus exerts a predominantly *inspiratory* augmenting influence.

Stimulation of the saphenous nerve increases both inspiratory and expiratory activity.

Carbon dioxide administration causes both a deeper inspiration and a more complete expiration.

Sodium carbonate apnea may occur at the normal expiratory volume or above it depending upon the relative dominance of inspiratory or expiratory activity.

Low oxygen administration results in an augmentation of inspiration but a less complete emptying of the lungs on expiration. Cyanide acts similarly, except that in cases where the hyperpnea is extreme there may be a second phase of expiratory augmentation, due probably to inspiratory fatigue.

Hemorrhage results in an increased expiratory lung volume possibly due simply to the decrease in volume of thoracic and abdominal contents.

REFERENCES

GESELL, R. Ergebn. d. Physiol., biol. Chemie u. experiment. Pharmakol. 43: 477, 1940.

GESELL, R. AND M. A. HAMILTON. This Journal 133: 694, 1941.

GESELL, R. AND C. MOYER. This Journal 131: 674, 1941.
          This Journal 135: 539, 1942.

SOBIN, S. AND H. C. NICHOLSON. This Journal 124: 491, 1938.

TURNER, J. M. This Journal 123: P97, 1938.

WORZNIAK, J. J. AND R. GESELL. This Journal 126: P658, 1939.

FETAL SURVIVAL FOLLOWING THE INJECTION OF ANTUITRIN-S IN PREGNANT RATS AND RABBITS

R. M. COCO

From the Department of Zoology, Physiology and Entomology, Louisiana State University, Baton Rouge

A number of investigations have been made concerning the effects of gonadotropic substances on pregnancy. Antuitrin-S injected during the first half of the gestation period usually resulted in the birth of normal fetuses at term in rats and rabbits (1, 2). Prolonged gestation and postmature fetuses were obtained by the administration of crude anterior pituitary and urine extracts (3–6) and Antuitrin-S (7–12) in late pregnancy, but more recent work on rats with Antuitrin-S (13) and human pregnancy serum (14) did not substantiate these results. This report is the result of a more intensive and extensive study of the effects produced on the fetuses by injections of Antuitrin-S into pregnant rats and rabbits.

METHODS. Eighty-six rats and 16 rabbits were used in this study. The methods employed with rats were the same as those discussed in an earlier paper (13) and need not be repeated here. The rabbits were mated in the early morning of the first day of gestation (the gestation period being 32 days). The condition of the fetuses was ascertained by autopsy, laparotomy, or by their appearance at birth.

RESULTS. *Rats.* Fifteen animals were injected with 40–100 R. U. of Antuitrin-S on the 5th–19th day of gestation. At autopsy on the 15th, 17th, 18th, 19th or 20th day, 112 fetuses were recovered. Seventy-four and one-tenth per cent of these were living and 25.9 per cent were dead.

Seventy-one animals were injected with 40–200 R. U. on the 14th, 15th, 16th, 17th, 18th, 19th, 20th or 21st day of pregnancy. Six hundred and twenty-three fetuses were obtained from these animals. One hundred and thirteen were born prematurely; 98.3 per cent of these being dead and 1.7 per cent alive. Three hundred and twenty-eight fetuses were born at term—53.1 per cent were dead and 46.9 per cent alive. Those born past term numbered 33–51.6 per cent were dead and 48.4 per cent alive. The condition of 149 fetuses—68 before term, 66 at term and 15 past term—was obtained at autopsy or by exploratory laparotomy on the 18th, 19th, 20th, 21st, 22nd, 24th or 26th day. Of those examined before term, 17.6 per cent were dead in utero and 82.4 per cent were alive; 47.0 per cent of those examined at term were dead and 53.0 per cent alive; those examined past term were all dead. Of the total number of fetuses (623), 16.8 per cent were viable and 83.2 per cent non-viable. Three hundred and one of the fetuses were from animals injected on the 18th day and of this number only 3.6 per cent were viable. It is evident that the fetuses were most adversely affected when the injections were made on the 18th day. It may be said

generally that two factors were paramount in determing the viability or non-viability of the fetuses—dosage and time of injection. The smaller doses (50–75 R. U.) usually resulted in birth occurring prematurely or at term and the larger doses (100–200 R. U.) in postponed and prolonged parturition. With the exception of one possible case the fetuses showed no signs of post-maturity.

Rabbits. Sixteen rabbits were injected intravenously with 40 R. U. of Antuitrin-S per kilogram of body weight on the 23rd, 24th, 25th, 26th or 27th day of pregnancy. Eighty-one fetuses were obtained from this group. In no case did parturition occur prematurely or at term and in 2 cases it occurred past term. One animal resorbed her young and in one case one fetus was retained in utero 81 days (49 days past term). Living fetuses were born to 1 animal on the 33rd day. The condition of the remainder of the fetuses ascertained by autopsy was as follows: 15 fetuses, 13.3 per cent dead and 86.7 per cent alive, at term; 15 fetuses, 60.0 per cent dead and 40 per cent alive, before term; and 45 fetuses, 84.4 per cent dead and 15.6 per cent alive, past term.

The ovaries of practically all rats and rabbits injected with Antuitrin-S were enlarged and contained induced corpora lutea in various stages of development; large luteal cysts, some of which were hemorrhagic, were not uncommon. In several instances very large follicles were present, most of these being cystic and atretic and an occasional one hemorrhagic.

DISCUSSION. A review of the literature revealed that the reported prolongation of pregnancy obtained by the injection of progestin, human pregnancy urine, and anterior pituitary extracts is usually attributed to the secretion of the corpora lutea administered as an extract or secreted by the corpora lutea induced in the ovaries of the experimental animals. It is significant that in this study 48.7 per cent of the rats and 43.8 per cent of the rabbits injected in late pregnancy had induced corpora lutea in the ovaries but these were unable to maintain viable fetuses past term.

Since there was a high mortality rate of fetuses in utero before or at term and a low incidence of living, postmature fetuses, it appears that Antuitrin-S acted as an abortient rather than an agent prolonging gestation. Histological studies of the placentae and uteri of animals bearing dead fetuses in utero revealed, in most cases, hemostasis, infarct formation and necrosis. It was evident that the blood supply through the placenta had been decreased, and it is possible that this, plus excessive uterine pressure, were factors involved in causing the death of the fetuses in utero. This latter factor is partially supported by the fact that some fetuses were compressed at birth.

SUMMARY

Varying amounts of Antuitrin-S injected into pregnant rats and rabbits in late pregnancy usually resulted in death of the fetuses in utero or soon after parturition and rarely in postponed and prolonged parturition. The ovaries usually contained induced corpora lutea but these evidently did not reach a threshold level of secretion.

REFERENCES

(1) KING, J. L.   This Journal **122:** 455, 1938.

(2) WISLOCKI, G. B. AND L. GOODMAN.   Anat. Rec. **59:** 375, 1934.

(3) TEEL, H. M.   This Journal **79:** 170, 1926.

(4) EVANS, H. M. AND M. E. SIMPSON.   Proc. Soc. Exper. Biol. and Med. **26:** 845, 1929.

(5) LEVIN, L., P. A. KATZMAN AND E. A. DOISY.   Endocrinol. **15:** 207, 1931.

(6) D'AMOUR, F. E. AND M. C. D'AMOUR.   This Journal **105:** 26, 1933.

(7) SNYDER, F. F.   Bull. Johns Hopkins Hosp. **54:** 1, 1934.

(8) SNYDER, F. F. AND M. ROSENFELD.   This Journal **119:** 153, 1937.

(9) HOOPES, E. C. AND J. L. KING.   This Journal **111:** 507, 1935.

(10) HOOPES, E. C.   Proc. Soc. Exper. Biol. and Med. **31:** 1115, 1934.

(11) HAIN, A. M.   Quart. J. Exper. Physiol. **25:** 131, 1935.

(12) KOFF, A. K. AND M. E. DAVIS.   Am. J. Obst. and Gynec. **34:** 26, 1937.

(13) COCO, R. M.   Endocrinol. **26:** 1057, 1940.

(14) ROSENFELD, S. S., B. LAPAN AND H. BARON.   Endocrinol. **29:** 276, 1941.

THE PHYSIOLOGY OF THE EMBRYONIC MAMMALIAN HEART BEFORE CIRCULATION

C. M. GOSS

From the Department of Anatomy, University of Alabama

The embryonic heart beats for several hours before it brings about the circulation of the blood. During this interval there is an elaboration of the contractile activity which results in a coördinated functioning organ. Since the various steps and phases of this development make their appearance more or less independently they can be studied separately or in a simplified form. The purpose of this paper is to describe this activity of the early embryonic heart and to present some conclusions which are significant for the interpretation of adult cardiac function.

The literature of historical interest and the recent experimental work dealing with the early development of the heart have been reviewed by Patten and Kramer (10). References to the literature of more immediate application will be made in the discussion.

METHODS. The observations were made on the hearts of rat embryos in tissue cultures. Whole embryonic vesicles were explanted, however, instead of fragments of tissue (5). Although the conditions inside the uterus could only be approximated by this technique the results show that the embryos were mounted in a reasonably favorable environment. The temperature was maintained at $38\pm1°C$. and mechanical agitation cut to a minimum by keeping the cultures in an incubator built especially to house the microscope which was used for observations. A mixture of 80 per cent oxygen and 20 per cent air was introduced into the moist chambers because it was found that it stabilized the contraction and prolonged the activity. The embryos remained in vigorous condition and continued their morphological development for 18 to 24 hours and the hearts contracted for 3 to 4 days without change of medium.

The litters of 53 rats with a total of 527 embryos were used. Of these 292 were successfully installed in cultures. The heart rate was counted on 138 embryos and cinematographic records were made of 48. Preserved embryos were used for reference and for checks of the age of embryos and number of somites. The fixed material included serial sections of at least 15 embryos for each number of somites between 2 and 9.

For the morphology of the heart of the rat embryo at various stages the reader is referred to the excellent stereoscopic pictures of Burlingame and Long (1). Although a considerable variation between the degree of development of the heart and the number of somites has been given by most investigators of the subject (including Goss) (5), in this paper a definite relationship between heart and somites will be maintained. Our reason for doing this is that a close correspondence was found in serial sections of fixed embryos where the number of

somites could be counted accurately, and we suggest that experimental error may enter into the counting of somites of whole embryos either fixed or alive.

The age of the embryos will be given in somites, therefore, as the most satisfactory unit for measurement of developmental time. The statistical evidence indicates that the formation of each of the first nine somites requires 2 to 3 hours of intrauterine life. Although contraction of the heart continued apparently unabated in many of the cultures, the development of morphological units such as the somites was definitely retarded. By comparing embryos of different ages immediately after removal from the uterus with embryos which had been in cultures for varying periods of time, it was found that developmental processes took roughly twice as long in culture as in the uterus.

RESULTS. Initiation of contraction occurred in embryos with three somites or approximately 9 days and 14 hours old. The heart at this stage is composed of two lateral rudiments separated from each other by the anterior-intestinal portal (5). The first visible activity was the feeble twitching of a few cells in the myocardial mantle of one heart (the left) just to the ventricular side of the atrio-ventricular junction. The endocardium in this region had formed a tube about the size of an adult capillary. These first contractions had a regular rhythm and a surprisingly constant rate of between 34 and 42 beats per minute (table 1).

The beginning of contraction in the heart rudiments of the other (the right) side was seen about two hours later. It commenced in the ventricle near the atrio-ventricular junction, was regular but somewhat slower than the left and completely independent of it. Both heart rudiments continued to contract independently for 2 or 3 hours or through the period when the embryo had four somites. The activity spread in the splanchnic mesoderm, the layer from which the myocardium is formed, until the two lateral hearts became distinct tubular structures. During each beat the contraction spread as a "peristaltoid" wave (10) from the venous or atrio-ventricular end toward the arterial end. Each heart had its own characteristics. The left was more curved and saccular, the right more narrow and straight. The left had a more rapid rate and contracted with a snap. The right was slower and the peristaltoid wave appeared to give a more powerful squeeze. These differences have been exaggerated experimentally by making the two rudiments continue their development as independent organs instead of allowing them to become incorporated in the normal single ventricle (4).

Although the contractile layer of each lateral heart was visible as a distinct fold, its continuity with a larger sheet, the splanchnic mesoderm, was clearly demonstrable also. The latter, as the ventral wall of the cleftlike pericardial cavity, arched around the cephalic end of the embryo linking the heart primordia of the two sides into a single structure with the shape of the letter U. As the lateral hearts developed they increased in size by incorporating more and more of this intervening sheet in their contractions. Finally the contractions involved the layer up to and across the median plane to establish a single saccular ventricle which extended over the foregut. This occurred at about the time the embryo formed the fifth somite.

The entire heart contracted as a unit once the junction was accomplished. The contraction wave began at the atrio-ventricular junction of the left side and spread in a peristaltoid wave to the junction of the right side. It did not travel from venous to arterial opening as one might expect or as it did in the lateral hearts before their union.

The myocardium developed until it almost completely surrounded the endocardium to form a single squat tubular ventricle during the period when the embryo had 6 somites. The shape of the heart was such, however, that the right and left atrio-ventricular regions were not as widely separated as before and the contraction wave appeared to travel from the venous to the arterial end of the tube rather than from side to side as in the preceding stage. The pace making activity of the left side became less stable and not infrequently the right side set the pace.

When the atrium began contracting there was no structural evidence that a new center had appeared. Its presence was not appreciated, therefore, in direct observations with the microscope. The earliest activity was discovered in slow-motion cinematographs. A small group of cells just on the atrial side of the atrio-ventricular junction contracted at a definite interval, measured 0.1 to 0.2 second on slow motion pictures, before the rest of the heart. Direct somite counts were not entirely satisfactory but they indicated that this took place toward the end of the 6 somite stage. The atrium was recognizable morphologically and the pause distinguishable without the aid of cinematographs in most 7 somite embryos. Once the atrium became active it was pace-maker for the whole heart. Occasional short periods of intermittency were observed but it is not known whether these were the manifestation of unstable activity or the result of injury. The ventricle at this time had acquired a partial S curvature. Its contraction gave a vigorous squeeze which completely closed the lumen of the endocardium and appeared more competent to propel the blood in circulation.

The bilaterality of the early heart was again evident at the time of initiation of contraction in the atrium. It would be more accurate to speak of two atria but we hesitate to call them right and left atria because they are not the direct forerunners of those chambers in the adult. The slight constrictions of the heart tubes which we have designated the atrio-ventricular junctions were still in evidence as limiting boundaries between the ventricle and the venous tubes which diverged abruptly at the anterior intestinal portal to run laterally toward the yolk sac. In the majority of cases the atrial region of the left side was the pacemaker for the whole heart, and although such activity was seen on the right side, it seems likely that the left side is the normal pacemaker in the early atrium. In certain embryos which had apparently been injured the atrial rudiments acted as independent centers, setting the pace for portions of the ventricle on their own sides. In one of the cases of double heart which developed after suppression of the median region of the embryo (an experiment performed later than those reported by Goss (4)) each of the widely separated hearts had a structurally distinct atrium which set the rhythm for its own ventricle.

Circulation of the blood began in embryos with 8 somites. Prior to this there had been backward and forward motion of blood cells in the yolk sac vessels or of the occasional free cells seen in the aorta and heart. As the time for circulation approached, the cells in the vitelline veins moved a little farther toward the heart than they did back again. Finally a complete circuit was established in the endothelial tubes and blood cells progressed haltingly into the heart. At first there were only a few cells in the circulating fluid, but the number increased rapidly as more were washed out of the yolk sac capillaries. The atrium had acquired distinct morphological outlines at this time but its physiological significance was confined to its power as pacemaker, since it appeared to contribute nothing to the mechanical pumping. The period of time between the initiation of contraction and the beginning of circulation is from 12 to 15 hours.

TABLE 1

NUMBER OF SOMITES	REMARKS	NUMBER OF CASES	AVERAGE RATE	RANGE OF RATES	S.D.
				per min.	
3	Left heart (initiation of contraction)	13	38	34–43	2.9
3	Right heart (first contraction*)	4	31	30–32	1
4	Left heart	11	47	38–59	5.6
4	Right heart	4	42	31–53	
4	Ventricle single	15	46	37–52	4.5
5	Ventricle	24	53	43–63	4.8
6	Ventricle	21	67	60–78	5
6	Beginning atrium	4	69	64–71	3
7	Atrium pacing	12	74	66–80	4.1
8	Atrium pacing	11	87	74–99	5.7
8	Beginning circulation	4	79	64–92	12

* This means first contractions observed on right side, not first contractions in the embryo.

The data on contraction rates summarized in table 1 were selected as the most reliable and significant. Those eliminated were principally the records of embryos which showed signs of injury or abnormality. Some inaccurate counts may have been included but it seemed desirable to retain as large a number as possible. The averages agree reasonably well with the most vigorous and carefully observed individuals, those which would be chosen as typical cases. The greatest error was due to the difficulty in counting the somites. It was made more accurate in the later experiments by carefully checking the number of somites with the morphological development of the rest of the embryo, particularly the heart and forebrain. The typical stages of the latter were established by wax plate reconstructions of fixed serial sectioned embryos. Nearly all of the counts were made within three hours of removal from the uterus and many of them represent averages of two or more counts made at 15 to 30 minute intervals.

The increase in heart rate during development is a gradual one and is not

marked by the jumps which are suggested by the average values in the table. By a coincidence the rate per minute is approximately ten times the number of somites up through eight and a useful representation would be, for example, 4 somites, 40 to 50; 5 somites, 50 to 60; and so on. There is an obvious overlapping of rates from one somite age to then extand the data are not, therefore, statistically significant except as they show the trend. The standard deviations are included because they give information on the scattering of values.

DISCUSSION. Spontaneous contractions occur when the heart is still rudimentary. They have a regular rhythm in Amblystoma (2) and in the rat (5) but there is a disagreement concerning this point in the chick. They are regular according to Sabin (11) and Johnstone (8) but Patten and Kramer (10) using a cinematographic recording method found that irregular twitchings preceded the regular contractions. All these authors agree that the ventricle contracts first; in fact, the heart is composed entirely of ventricle at this stage. Copenhaver (2) proved conclusively that the ventricle initiates the contraction by first marking the contractile cells with a vital dye and then later identifying them in the ventricular wall after the heart had developed for several days.

Contraction of the two primitive lateral hearts seems to be peculiar to mammalian embryos and is an indication of the precocious development of the vascular system in this class. The first contractions in the chick (10) and in Amblystoma (2) occur in the single median ventricle. The contracting lateral hearts are more widely separated in the rabbit than in the rat (3), and they remain apart longer, even after the atria have developed. The early morphological differences between the two lateral hearts reported in mammals other than the rat and the constancy of physiological differences found in living embryos (3, 5) show that an asymmetry in the heart is established at a very early period.

The left heart was given above as the site of initiation of contraction although contractions were occasionally seen on the right side first. The latter were observed only in embryos in which the left heart never contracted, apparently because of injury (5). Initiation of contraction on the right side has not been ruled out as a possibility, therefore. The right side of the single ventricle has been given as the position of first contractions in the chick (10) but neither side was found to predominate in Amblystoma (2).

A progressive wave of contraction, called peristaltoid by Patten (10), is characteristic of the heart muscle almost from the first. It travels from venous to arterial ends of the lateral hearts, from left to right in the single ventricle in its saccular stage and from atrial to aortic ends of the S shaped tube at the beginning of circulation. This wave, obliterating the endocardial lumen as it progresses, operates very effectively in the absence of valves to propel the blood out of the early heart.

Two of the most important factors in coördinating the chambers of the heart seem to be inherent in the myocardium itself. These are first, the pause between the atrium and ventricle, and second, the ability of one chamber to inhibit the spontaneous activity of a neighboring chamber. In its earliest stages the atrium was recognized as a center of activity by the pause between its contraction

and that of the ventricle.   The atrio-ventricular interval became somewhat longer during the period of development under observation but it approximated the adult value.   At this stage the contractile cells of the atrium are in close contact with those of the ventricle.   Intervening tissue which might act either as an insulating medium or conduction system has not been identified (10). Copenhaver (2) and Patten and Kramer (10) do not mention the interval and they apparently used morphological appearance alone for identification of the early atrium.

Although the ventricle has the power to originate its own rhythm in the early stages, once the atrium becomes pacemaker it seems to have the power to inhibit the spontaneity of the ventricle.   In our experiments heart block was frequently seen in embryos which had been in culture for many hours but it was a partial, not a complete block.   In still older cultures the contraction was intermittent but the ventricle did not become independent of the atrium unless a mechanical injury separated the two chambers.   A cut or a ligature, of course, does allow the ventricle to revert to a spontaneous rhythm at its own intrinsic rate (2, 10, 8).   The ability to be inhibited would seem to be a fundamental property of all the chambers of the heart if we consider the action the same in the inhibition of the ventricle by the atrium, the atrium by the sinus and the sinus by the vagus nerve.

Copenhaver (2) has shown by his very careful and complete experiments with Amblystoma embryos that the chambers of the heart begin contracting at their own intrinsic rates.   Our observations are confirmatory as far as we have been able to carry them.   The contraction rate of the whole heart in rat embryos increases gradually, that is, there is no sudden jump when the atrium becomes active.   When the rate reaches a value of approximately 69 (table 1) the atrium takes over the pace-making function.   Rates above this value in ventricle alone were rarely seen.   The lowest rate recorded for the atrium was 53 per minute, but this low value was seen only in embryos which had been in cultures for more than six hours.   A few preliminary observations indicate that the sinus activity begins at a rate above 120.   It will be necessary to perform experiments similar to Copenhaver's of cutting the ventricle from the atrium in older embryos in order to determine the intrinsic rates precisely.   Preliminary values may be given, however; for the ventricle between 40 and 70 and for the atrium between 70 and 120.   The latter may seem to be a wide range and a high value but it must be recalled that the average rate for an adult rat is 458 per minute (7).

Physiological activity precedes structural differentiation in both the ventricle and the atrium.   This applies to the morphological development as well as to the cytological differentiation.   In both chambers, the cells which contract first are not marked off from the rest of the splanchnic mesoderm in that region except by their topographical position in relation to the future atrio-ventricular junction.   The cytological differentiation of fibrillae with cross striations appears after the muscle cells have been contracting for several hours and the circulation is established (6, 2).   The early hearts were fixed according to the method recommended by Lewis (9) while they were contracting and the effect watched under

the microscope.   These findings make it difficult to accept theories of muscular contraction which are based on the presence of specialized cytological structures such as cross striations.

The beginning of circulation marks the climax of a concerted development on the part of the embryo.   The heart has developed into an adequate pumping mechanism, the endothelium has established a complete circuit of hollow tubes and the cells in the blood islands have elaborated hemoglobin to a point where they are effective carriers of oxygen.   Although the establishment of the complete circuit seems to take place rather abruptly, the effect of the beating heart can be seen before this time by the backward and forward motion of occasional cells in the lumen of the heart or vessels.   It is probable that this produces a kind of seeping circulation which serves to move the fluids and improve the exchange of materials both within the embryo and between the embryo and the maternal reservoirs of blood surrounding the yolk sac and membranes.

CONCLUSIONS

The following points concerning the fundamental or intrinsic powers of the myocardium have been made in our observation of early embryonic hearts: First, the power of spontaneous rhythmical contraction is possessed by each of the chambers and they have their own intrinsic rates.   Second, the contraction of the myocardium progresses by a wave from one end of the chamber to the other.   Third, the atrio-ventricular interval which makes coördination of the whole heart possible appears along with atrial contraction itself.   Fourth, the spontaneous rhythm of the ventricle is inhibited by the atrium.   Fifth, mechanical work, pumping the blood, begins after the preparatory development outlined above.

REFERENCES

(1) BURLINGAME, P. L. AND J. A. LONG.   Univ. of Cal. Publications in Zoology 43: 249, 1939.
(2) COPENHAVER, W. M.   J. Exper. Zool. 80: 193, 1939.
(3) DWINNELL, L. A.   Proc. Soc. Exper. Biol. and Med. 42: 264, 1939.
(4) Goss, C. M.   J. Exper. Zool. 72: 33, 1935.
(5) Goss, C. M.   Anat. Rec. 70: 505, 1938.
(6) Goss, C. M.   Anat. Rec. 76: 19, 1940.
(7) HAMILTON, J. B., M. DRESBACH AND R. S. HAMILTON.   This Journal 118: 71, 1936.
(8) JOHNSTONE, P. N.   Johns Hopkins Hosp. Bull. 36: 299, 1925.
(9) LEWIS, M. R.   Johns Hopkins Hosp. Bull. 30: 176, 1919.
(10) PATTEN, B. M. AND T. C. KRAMER.   Am. J. Anat. 53: 349, 1933.
(11) SABIN, F. R.   Contrib. to Embryol. 9: 213, 1920.

THE EFFECT OF HYDROCHLORIC ACID ON THE PYLORIC SPHINCTER, THE ADJACENT PORTIONS OF THE DIGESTIVE TRACT AND ON THE PROCESS OF GASTRIC EVACUATION[1]

J. P. QUIGLEY, M. R. READ, K. H. RADZOW, I. MESCHAN AND
J. M. WERLE

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

Despite numerous attempts to determine the influence of hydrochloric acid on the motor activity of the pyloric sphincter and the immediately adjacent portions of the gut and on the process of gastric evacuation, uniformity of opinion has not been attained. The literature in this field has been reviewed especially by Cannon (1), Babkin (2), Alvarez (3) and Van Liere and Sleeth (4).

According to a popular concept, gastric evacuation is chiefly controlled by pyloric sphincter activity and this in turn is largely regulated by HCl; acid in the stomach causing sphincter relaxation and gastric evacuation; acid in the duodenum delaying emptying by producing pyloric spasm. The theory has repeatedly been questioned, but the investigations favoring or opposing it largely involved studies supplying only indirect information regarding sphincter activity; chiefly fluoroscopic observations or the measurement of volumes of material expelled from a duodenal fistula or recovered from the stomach. A direct method of studying sphincter and antral activity was employed by Thomas, Crider and Mogan (5) and led them to conclude that acid in the duodenum caused a pylorospasm followed by inhibition of the sphincter and antrum.

This problem has not been investigated in the normal trained dog by the multiple balloon method and the fluoroscopic-optical manometer technic, despite the fact that these methods appear particularly adaptable to the problem. We have therefore undertaken this type of investigation.

Balloon studies, such as we have employed in this study, permit *direct* observations of the motor activity of the entire pyloric sphincter region. The investigation was also designed so definite volumes of acid in known concentrations came directly into contact with the portion of the gut whose susceptibility was to be tested. Since in fasting animals, the acid would encounter no food and little secretion, the rate of adsorption or neutralization should be moderate. An additional reason for employing fasting animals for many of the experiments is dependent on the fact that previous studies have shown the motility of the pyloric sphincter region prevailing during fasting is particularly susceptible to modification by experimental procedures.

Seven dogs provided with cannulae affording access to the stomach and duodenum and trained to co-operate with the experimental procedures were employed. While comfortably resting on mattresses, three tandem balloons were

introduced by the method of Meschan and Quigley (6) to lie in the pyloric sphincter and immediately adjacent to it in the antrum and bulb. A fourth balloon was placed in the mid-duodenum. Employing basal pressures of 2 cm. of water in the systems, records were made from water manometers. The experiments were started 18 hours post-cibum. The test solutions at body temperature were administered on each occasion over an interval of two minutes, while vigorous, uniform motility was in progress in all four regions.

Acid in the stomach. Hydrochloric acid was introduced into the pyloric antrum by a tube opening 4 cm. proximal to the sphincter. We have found that substances so given rapidly bathe the entire antrum and usually enter the duodenum after an interval of 4 to 6 minutes. Administration of 10 cc. quantities of 0.2 per cent HCl on ten occasions produced no modification in motility of the sphincter region or mid-duodenum on eight trials, but was followed by a slight augmentation in tonus limited to the antrum on the two remaining trials. Administration of 5 cc. quantities of 0.4 per cent HCl in eighteen experiments produced no modification on sixteen occasions, but in two trials resulted in a slight rise in tone of the antrum, sphincter and bulb; 10 cc. quantities of 0.4 per cent HCl used in eight experiments produced no effect on five occasions and slightly augmented the tonus of the antrum and sphincter in three cases. The results of these 34 experiments justify the conclusion that under the conditions here employed, hydrochloric acid in the antrum certainly does not relax the pyloric sphincter and usually was without any effect on the sphincter region.

Acid in the duodenum. In a manner similar to that described above, injections into the duodenum were made through a tube terminating in the first portion of the lower third of the duodenum. The sole effect from the introduction of 10 cc. quantities of 0.2 per cent HCl in eight experiments was a depression of the motility and tone of the antrum, sphincter and bulb. A preliminary period of stimulation did not obtain. The latent period averaged 1.5 minutes in the antrum, two minutes in the sphincter and four minutes in the bulb. The inhibition persisted for an average of 6 minutes in the antrum, 3 minutes in the sphincter and two minutes in the bulb. The record from the mid-duodenum, just proximal to the site of acid injection showed a moderately augmented tone and motility during the last minute of the acid administration period and subsequently a two minute period of inhibition.

An attempt to simulate the manner in which hydrochloric acid might enter the duodenum under physiological conditions was made on five occasions. Hydrochloric acid in 2 to 3 cc. quantities of 0.2 per cent HCl was repeatedly introduced into the distal duodenum at 3 to 5 minute intervals. Each injection of acid produced a moderate depression of the antrum and sphincter, but motility in the bulb and mid-duodenum usually were unaltered. A much more profound inhibition of the entire sphincter region followed the administration of similar volumes of oleic acid (fig. 2).

The results obtained from the introduction of 10 cc. quantities of 0.4 per cent HCl into the distal duodenum (11 expts.) were similar to but more marked than those described above from the same volume of 0.2 per cent HCl. The inhibi-

tion developed in the antrum, sphincter and bulb in 2, 3 and 4 minutes and persisted in these regions for 11, 8 and 4 minutes respectively (fig. 1). A

Fig. 1. Tandem balloon—water manometer record—taken 18 hours post cibum. At A, administered 10 cc. 0.4 per cent hydrochloric acid into the distal duodenum.

Fig. 2. Tandem balloon—water manometer record—taken 18 hours post cibum. Injected into distal duodenum at A, 5 cc. 0.2 per cent HCl; at B, 3 cc. 0.2 per cent HCl; at C, 2 cc. 5 per cent oleic acid; at D, 3 cc. 5 per cent oleic acid.
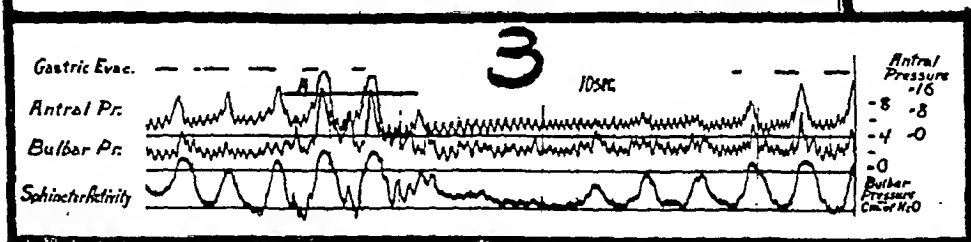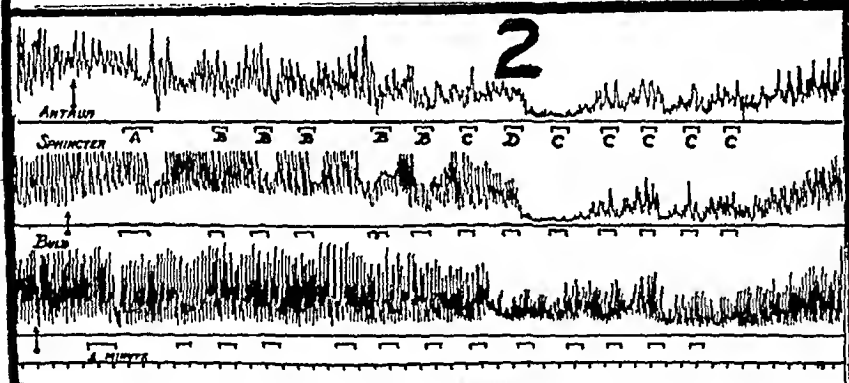
Fig. 3. Animal fed corn meal mush and $BaSO_4$. At A, 10 cc. 0.4 per cent hydrochloric acid was administered into the proximal duodenum. Records made by fluoroscopic observations, optical manometers and miniature sphincter balloon (downward movement of sphincter record indicates sphincter relaxation).

slight, transient augmentation of tone and motility in the sphincter region during the later portion of the administration period preceded the period of inhibition on three occasions.

Thus, direct evidence shows that under the conditions of these experiments the presence of HCl in the distal duodenum inhibits the entire sphincter region. This would indicate that the retardation of gastric evacuation produced by hydrochloric acid is primarily due to suppression of the antrum and not to any important involvement of the sphincter and bulb.

Boldyreff (7) and many others have noted a regurgitation of duodenal contents related to the presence of acid in the sphincter region. Our observation that acid in the duodenum produced an inhibition which was most pronounced in the antrum and became progressively less marked in the more distal structures would suggest this regurgitation resulted from a reversal of pressure gradient so the bulbar pressure exceeded the antral pressure. The intermediation of reversed peristalsis would not be essential to this regurgitation. Antiperistalsis may further be ruled from participation in this phenomenon since when it is present it is definitely recognized by our experimental method, but in these studies no indication of such reversed motility was encountered.

Optical manometer-fluoroscopic studies were undertaken to determine the influence of hydrochloric acid administration on the pressures developed in the antrum and bulb and the process of gastric evacuation. These studies should also serve to check on certain inferences drawn from the first portion of this investigation. In the analysis made by Werle, Brody, Ligon, Read and Quigley (8), gastric emptying was shown to occur in cycles, during phase A; the last portion of the period antral basal pressure exceeded bulbar basal pressure, and during phase B, the first portion of the antral phasic pressure wave. We recorded these pressures from open tip tubes placed 18 mm. apart, one in the antrum and one in the duodenal bulb. The optical manometer technic described by Brody, Werle, Meschan and Quigley (9) was employed in normal trained dogs during both the fasting and fed state. In some additional experiments the pyloric sphincter activity was studied simultaneously with the pressure registration by placing a tiny balloon (3 x 8 mm.) in the sphincter lumen, and likewise arranging for registration by an optical manometer. In the fed animals, rapidly repeated fluoroscopic observations were combined with the pressure registrations.

Animals 18 hours post-cibum. In 15 experiments, 10 cc. of either 0.2 or 0.4 per cent HCl was injected into the duodenal bulb *immediately* distal to the sphincter, i.e., in the region where gastric contents normally first encounter the duodenal mucosa. This produced a cessation of phasic pressure waves in the entire sphincter region. The antral inhibition started 10 to 30 seconds after beginning the injection and persisted for 1 to 4 minutes, the bulb was inhibited for 40 to 110 seconds and the sphincter (records made only in 8 cases) for 60 to 140 seconds. The antral basal pressure fell more than the bulbar basal pressure, thus the conditions for gastric evacuation terminated, since both phases A and B were suppressed. In two experiments, chiefly those involving the injection of 0.4 per cent HCl, a very transient excitation consisting of 3 to 4 rapid and

vigorous antral pressure waves preceded the inhibition phase and in eight trials a similar excitation of the bulb began 5 to 10 seconds after the injection started and persisted for 10 to 30 seconds. Control injections of 10 cc. of Ringer's solution into the bulb sometimes produced a similar but much more moderate stimulation of the sphincter region, but without the subsequent inhibition.

Fed animals. In a series of 18 experiments similar to those just described, the animals were fed through the gastric cannula 500 cc. of corn meal mush containing 80 grams $BaSO_4$. While fluoroscopic observations showed that active gastric evacuation was in progress, 10 cc. quantities of either 0.2 or 0.4 per cent HCl was injected into the proximal duodenum, figure 3. Antral pressure waves were completely abolished for 1–3 minutes and no antral peristaltic waves were visible fluoroscopically. Gastric evacuation ceased with the last antral peristaltic wave and the last antral phasic pressure wave. Inhibition of the bulb and sphincter was also the usual effect. The duration of this inhibition was approximately 120 seconds in the antrum and 90 to 70 seconds in the sphincter and bulb. A preliminary excitation of the antrum, sphincter and bulb occasionally preceded the inhibition, but was less frequently seen, was of shorter duration and more moderate in degree in fed than in fasting animals. As antral contractions and phasic pressure waves returned (re-establishment of evacuation phases A and B), gastric evacuation returned to normal after the second or third wave.

In order to determine the effect of the presence of hydrochloric acid in the more distal portions of the duodenum and also to prevent the acid being diluted and flushed away by the material discharged from the stomach into the upper duodenum, a series of 23 experiments was performed. The animals were fed the standard meal mentioned above and the acid was introduced into the distal duodenum. Constant slight suction was applied to the duodenal cannula in an attempt to collect all the material accumulating in the upper duodenum. This collection was facilitated by an inflated balloon placed in the duodenum just distal to the pancreatic duct papilla so as to interfere with the passage of proximal duodenal contents.

The administration of 10 cc. of 0.2 or 0.4 per cent HCl into the distal duodenum produced within 10 to 30 seconds, after beginning the injection, complete antral inhibition. Antral peristaltic waves, bulbar contractions, antral and bulbar pressure waves, antral and bulbar phasic pressure waves and the positive antral phasic gradients, particularly evacuation phases A and B, were absent for 1–5 minutes. Gastric evacuation ceased with the last antral wave and returned 10–15 seconds after the reappearance of the first antral pressure wave. The inhibition of the bulbar activity always began slightly later and the recovery began slightly earlier than the corresponding antral change. An augmentation of bulbar activity did not result from acid introduced into the distal duodenum of fed animals. Sphincter activity was studied in nine experiments by the miniature balloon method and in all cases a sphincter inhibition closely resembling that obtaining in the antrum was observed; stimulation of the sphincter did not occur. During the brief interval during which the antral

pressure dropped below bulbar level, i.e., while a negative antral-bulbar pressure gradient prevailed, a small portion of bulbar contents could be fluoroscopically seen returning to the antrum. It should be emphasized that it is acid in the duodenum rather than in the stomach, which induces the conditions leading to duodenal regurgitation. The emphasis has previously been incorrectly placed on acid present in the stomach.

DISCUSSION. The results of our direct studies disagree with the popular interpretations placed on the results obtained by more indirect methods. Under the experimental conditions of our experiments, moderate quantities of hydrochloric acid *in the stomach* have been shown to exercise no specific control on the pyloric sphincter or the immediately adjacent portions of the gut. Usually it had no effect, it never produced inhibition, but occasionally it led to a slight augmentation of motility as any other indifferent substance might do.

Primarily, hydrochloric acid *in the duodenum* inhibited the entire pyloric sphincter region. This inhibition was most marked in the pyloric antrum and progressively decreased in degree in more distal regions, but was still demonstrable in the third portion of the duodenum. Hydrochloric acid placed in the bulb was slightly more effective than when injected into the lower duodenum and the animals were more susceptible when fasting than after feeding.

It was indicated on theoretical grounds and subsequently demonstrated experimentally, that hydrochloric acid in the duodenum retarded gastric evacuation by inhibiting the motility ("pumping" action) of the antrum. The period of suppressed gastric emptying began with the disappearance of antral peristaltic waves, antral pressure waves and evacuation phases A and B and returned with their re-establishment. Gastric emptying ceased *despite* a simultaneous inhibition of the pyloric sphincter. The inhibition of the duodenum was less marked in degree and duration than the proximal structures.

While acid was being injected into the duodenum, a transient increase in the activity of the sphincter region sometimes developed. This was produced chiefly by strong acid present high in the duodenum. Since it frequently was absent, or was of moderate degree and magnitude, it appeared to be an action of secondary importance and it probably was in the nature of a defense response. Since it was most marked in the sphincter and bulb it would be effective in expelling the irritant from the upper duodenum, on certain occasions preventing its return into the stomach, or with slightly different conditions as mentioned above, resulting in regurgitation. Under similar conditions we have obtained the same type of reaction to the presence in the upper duodenum of fats, or less frequently, of Ringer's solution.

Our results and conclusions differ from those of Thomas et al., especially in respect to this preliminary augmentation period, for in our studies it was much less frequent ór of shorter duration and in general appeared to be of less significance. We are both of the opinion that under physiological conditions, hydrochloric acid exerts only a moderate regulatory influence on the process of gastric evacuation in comparison to many other naturally occurring factors and that the influence exerted by hydrochloric acid arises primarily from antral inhibition due to acid in the duodenum.

SUMMARY

Employing direct observational methods, we obtained evidence that hydrochloric acid *in the stomach* exerts little or no physiological action on the motor activities and the pressure changes in the pyloric sphincter region or on the process of gastric evacuation.

Hydrochloric acid *in the duodenum* is moderately effective in suppressing the pyloric antrum and thus retards gastric evacuation. The pyloric sphincter and the upper duodenum are also inhibited, but this is of slight importance in the evacuation process, although some duodenal regurgitation may result from the more complete inhibition of the antrum than of the duodenal bulb. Acid in the duodenum may produce a preliminary augmentation of motility in the sphincter region, but in our experience this is of minor importance since it was rare, transient and moderate in degree.

REFERENCES

(1) CANNON, W. B. This Journal 20: 283, 1907.
(2) BABKIN, B. P. Die Aussere Sekretion der Verdauungsdrüsen Julius Springer, Berlin, 1928, p. 801.
(3) ALVAREZ, W. C. An introduction to gastroenterology. Paul B. Hoeber, Inc., New York, 1939, p. 354.
(4) VAN LIERE, E. J. AND C. K. SLEETH. Am. J. Digest. Dis. 7: 118, 1940.
(5) MOGAN, C. J. AND J. E. THOMAS. This Journal 97: 546, 1931.
    THOMAS, J. E., J. O. CRIDER AND C. J. MOGAN. This Journal 108: 683, 1934.
(6) MESCHAN, I. AND J. P. QUIGLEY. This Journal 121: 350, 1938.
(7) BOLDYREFF, W. Quart. J. Exper. Physiol. 8: 1, 1914.
(8) WERLE, J. M., D. A. BRODY, E. W. LIGON, JR., M. R. READ AND J. P. QUIGLEY. This Journal 131: 606, 1941.
(9) BRODY, D. A., J. M. WERLE, I. MESCHAN AND J. P. QUIGLEY. This Journal 130: 791, 1940.

ACCLIMATIZATION TO LOW OXYGEN TENSIONS IN RELATION TO GASTRIC EMPTYING

J. CLIFFORD STICKNEY AND EDWARD J. VAN LIERE

From the Department of Physiology, School of Medicine, West Virginia University, Morgantown

That anoxia delays gastric emptying has been demonstrated by Van Liere *et al.* in dogs (5) and in human subjects (6). Whether or not this delay would persist if the animals were exposed to continuous or discontinuous anoxia, as far as we know, has never been studied. Such a problem is of timely interest. That gastric disturbances occur often in people who ascend to high altitudes is commonly known.

The generally recognized adaptations to anoxia are: the increase in the total ventilation of the lungs; the fall in alveolar carbon dioxide tension with the concomitant rise in alveolar oxygen tension and increase in blood alkalinity which is kept within bounds by excretion of alkalies by the kidney; the increases in the number of erythrocytes and in percentage of hemoglobin; and the increased cardiac output. These changes may be regarded as compensatory mechanisms, and their appearance cannot be accepted as evidence of complete acclimatization unless it can be ascertained whether or not they are adequate to meet the severity of the anoxia encountered. Obviously more fundamental criteria of acclimatization are needed which would be measures of bodily function and thus serve as indices of the adequacy of the adjustments. The present study was undertaken in order to determine the extent to which the stomach is involved in the adjustment to oxygen want.

METHODS. Normal gastric emptying time was determined fluoroscopically on five dogs following a meal of 10 grams dried bread, 20 grams ground lean beef and 50 cc. skim milk to which 15 grams barium sulfate were added. These values were controlled by keeping the dogs, after the meal, in the low pressure chamber (at normal atmospheric pressure) in which they were later exposed to lowered oxygen tensions. The weights of the dogs were maintained constant by feeding them a supplementary meal. They were in the same environment throughout the duration of the experiments except when in use.

The dogs were then exposed daily (except Sundays) to diminished oxygen tensions in a low pressure chamber (4). The length of the daily exposure was about 8 hours. The oxygen tension to which the dogs were first exposed was 100 mm. Hg (12,000 ft.). They were exposed to this tension until their gastric emptying time was normal. Dogs 1, 4 and 5 were then exposed to an oxygen tension of 86 mm. Hg (16,000 ft.) until attainment of normal gastric emptying time as before. Finally all the dogs were exposed to an oxygen tension of 80 mm. Hg (18,000 ft.), and except for dog 1 this was continued for about sixteen weeks.

At the end of this time the oxygen tension to which each dog appeared acclimatized (as judged by gastric emptying time) was determined. The gastric emptying time of each dog at that oxygen tension was then determined periodically (at approximately 10 day intervals) until normal gastric emptying values were no longer found after two or three trials.
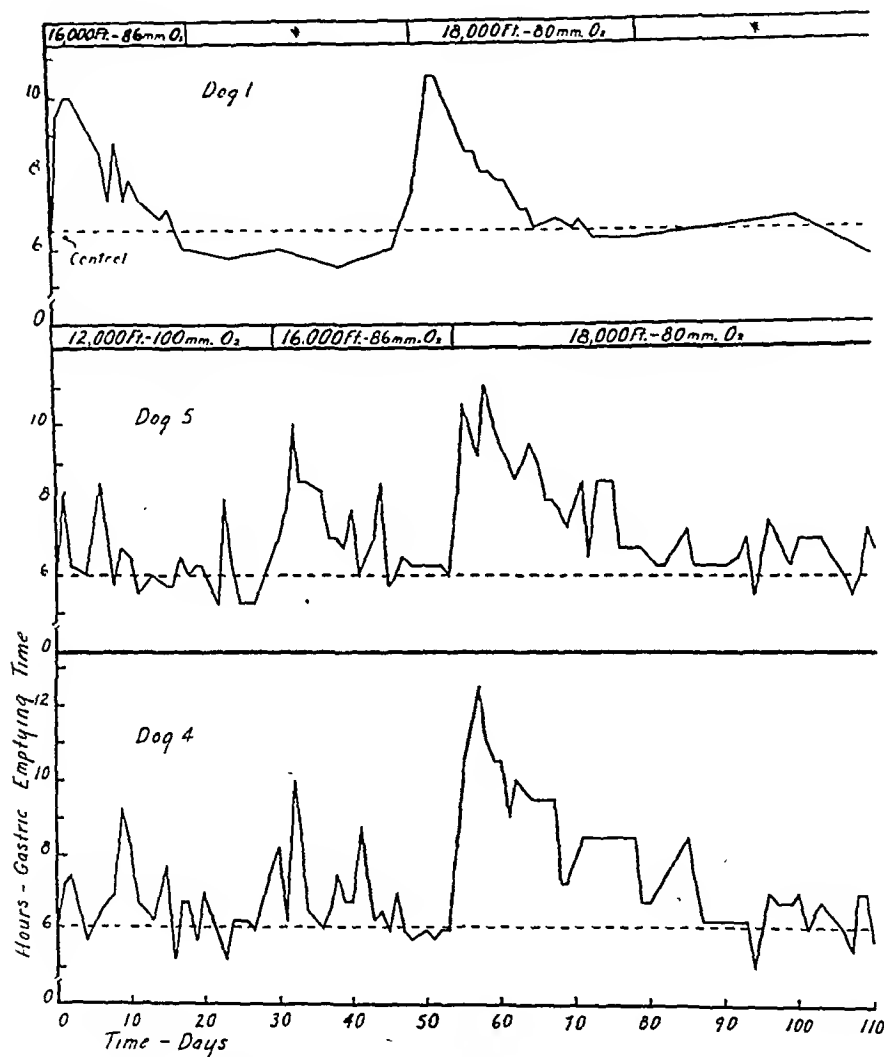
Fig. 1. Acclimatization to discontinuous exposure to anoxia

Throughout the study hemoglobin and red blood cell values were determined periodically.

RESULTS. The influence of discontinuous exposure to the three grades of diminished oxygen tension upon the gastric emptying time of three of the dogs is shown in figure 1. The results on the other two dogs were essentially similar but in order to save space the graphs are not shown. At 100 mm. O_2 the average delay (based on the greatest single delay in each dog) in gastric emptying in four dogs was about 35 per cent. The gastric emptying time of dogs 4 and 5 was

not affected as greatly by the anoxia as that of dogs 2 and 3. For this reason the quicker return to normal of dogs 4 and 5 was not unexpected. In dog 5 this apparently occurred near the beginning of the third week and in dog 4 somewhat later. This point was not reached in dogs 2 and 3 before the beginning of the eighth week.

At 86 mm. O_2 three dogs showed an average delay in gastric emptying of about 40 per cent. The return to normal occurred during the third week for dog 1 and during the fourth week for the other two dogs. For the next 30 days dog 1, exposed to this degree of anoxia periodically, showed less than normal emptying time on each trial.

An average delay in gastric emptying of about 60 per cent was obtained when the five dogs were exposed to an oxygen tension of 80 mm. O_2. The graphs show that upon daily exposure to this tension the gastric emptying time approached the normal. In dog 1 the normal was definitely reached during the third week. In the other dogs the normal was approached and in dog 4 temporarily reached, but subsequent results (not shown on the graphs) showed that it was not consistently maintained.

The retention of normal emptying time during anoxia after cessation of the period of daily exposure to the lowered oxygen tensions was quite variable, being over six months for dog 1 and less than a week for dogs 2 and 4.

DISCUSSION. That prolonged exposure to reduced oxygen tensions should result in adaptations whereby gastric emptying time is returned to the normal is not unexpected. In a personal communication to one of us (V. L.) Kerwin, without giving the details of his method, stated that in a series of fluoroscopic observations made on acclimatized residents of La Oroya, Peru, no delay in gastric emptying was noted. This is not surprising, because we found that our animals became reasonably well adapted in a relatively short time even though they were exposed to anoxic anoxia somewhat less than a third of the time.

Since it has been shown that the effects of anoxia on gastric emptying in dogs and human subjects are practically similar, one may apply, tentatively at least, the present results to men who are exposed to anoxia, such as aviators. In this connection it should be remembered that considerable individual variation in the ability to acclimatize exists.

The reason for the delay in gastric emptying produced by anoxia is not fully understood. Crisler and Van Liere (2) concluded that at moderate degrees of anoxia the cause of the delay is on a vagospastic-pylorospastic basis, and at greater degrees of anoxia there is further delay caused by the oxygen want directly affecting the gastric musculature. It is possible that other mechanisms producing delay may also be present. Thus the delay may be due to the effect of the anoxia upon the stomach itself or upon the complex of regulatory mechanisms to which the stomach is subject or to both. The return of gastric emptying to the normal during discontinuous anoxia as reported here means that an adaptation had taken place which was at least adequate for that phase of gastric function. In other words the thoroughness of the acclimatization has been measured by the return of normal function rather than by the extent of the

compensatory mechanisms. For this reason gastric emptying time may be considered as a fairly good objective measure of acclimatization.

It is of interest to consider the effect of the discontinuous anoxia on the hemoglobin and erythrocytes. There was a gradual rise in these values during the course of the experiments, and at the end of 24 weeks when the regular exposure to anoxia was terminated the average increase in four dogs was 74 per cent for hemoglobin and 84 per cent for erythrocytes. If one uses (as is generally done) these values as criteria in the experiments reported here, there is evident a pronounced degree of acclimatization.

Campbell (1) concluded on the basis of the pathologic changes as well as the physical fitness of his experimental animals that 20,000 ft. represented the ceiling for processes of acclimatization. For human beings McFarland *et al.* (3) cites evidence for a similar conclusion. The authors feel that the refractory results at 80 mm. O₂ (18,000 ft.) might be due to the nearness to the limit beyond which acclimatization is impossible.

SUMMARY

The gastric emptying time of five dogs after a standard meal has been determined during discontinuous exposure to reduced oxygen tensions (100, 86 and 80 mm. Hg corresponding respectively to simulated altitudes: 12,000, 16,000 and 18,000 ft.). The usual delay in gastric emptying produced by anoxia has been found to be gradually reduced in every case. The return to normal gastric emptying time has been complete under these conditions at 100 mm. O₂ in 4 dogs and at 86 mm. O₂ in 3 dogs. At 80 mm. O₂ but one dog of the five has shown complete return to normal gastric emptying time.

Considerable individual variation (from 3 to 8 weeks) has been found in the time for complete return to normal gastric emptying. The persistence of this adaptation to anoxia after the periods of discontinuous exposure had been ended has been from less than a week to over six months.

Since gastric emptying time, as an index of function, may indicate the adequacy of the adjustment of the compensatory mechanisms during anoxia, it has been suggested that it may be regarded as another criterion of acclimatization.

REFERENCES

(1) CAMPBELL, J. A. Brit. J. Exper. Path. **16**: 39, 1935.
(2) CRISLER, G., E. J. VAN LIERE AND I. A. WILES. Am. J. Digest. Dis. and Nutrition **2**: 221, 1935.
(3) McFARLAND, R. A. J. Comp. Psychol. **24**: 189, 1937.
(4) VAN LIERE, E. J. J. Lab. and Clin. Med. **21**: 963, 1936.
(5) VAN LIERE, E. J., G. CRISLER AND D. H. ROBINSON. Arch. Int. Med. **51**: 796, 1933.
(6) VAN LIERE, E. J., D. LOUGH AND C. K. SLEETH. Arch. Int. Med. **58**: 130, 1936.

TRANSMISSION OF RADIO-ACTIVE IRON TO THE HUMAN FETUS[1]

W. T. POMMERENKE, P. F. HAHN, W. F. BALE AND W. M. BALFOUR

From the Departments of Obstetrics and Gynecology, Pathology and Radiology of the University of Rochester School of Medicine and Dentistry

Evidence is presented below that iron can be recovered from the fetus within less than one hour after this same iron has been given orally to a pregnant woman. Such expeditious transfer speaks for a speedier mechanism than would probably obtain if this iron were first firmly incorporated in the hemoglobin molecule of the maternal red blood cell. With the discovery of radio isotopes, a sensitive method has been made available whereby it is possible to trace labeled particles of iron[2] in their journeys about the body, and to find them finally in their depots. The average requirement of iron for pregnancy is about 0.5 gram (7). Iron is required for the generation of fetal hemoglobin. Furthermore, large amounts of iron are stored in the fetal liver and other organs for subsequent use. Bunge (1) found that the liver of the rabbit at birth contains about 18 mgm. of iron per 100 grams of body weight, and only a corresponding 3.2 mgm. on the 24th day afterwards.. This endowment of iron with which the young is born constitutes a reserve which may be drawn upon later to compensate for the meager supply of iron in milk.

While blood constitutes about 7 per cent of the body weight, it contains about 60 per cent of the body iron. It would, therefore, seem a natural inference that whatever the immediate precursor of the fetal iron, a probable source is the iron in the maternal circulation. Another likely source is the iron reserve of the mother, notably in the liver. Diminution of the iron reserve in the spleen during pregnancy has been demonstrated by Charrin (2). The importance of the iron content of the mother's diet has been emphasized by Marshall (5) who noted that when the mother's diet is rich in iron, the fetus will store more iron than when the diet is poor in iron.

The precise manner of transfer of iron from mother to fetus still awaits demonstration. From early pregnancy to term, vessels in the villi bring fetal blood into close proximity to a liberal supply of iron, *i.e.*, the decidual pools. While it might be difficult to conceive that the barriers presented by the walls of the blood vessels and adjacent tissues would permit the passage of red blood cell encapsulated iron from mother to child, this close proximity would appear to meet the requirements of an osmotic connection. Tips of the villi may actually project into the maternal blood vessels. Stander (9) states that the fetus takes up iron from degenerated maternal erythrocytes through the syncytial covering of the villi. Scholten and Veit (8) observed that the chorionic villi are quite

capable of dissolving erythrocytes of the circulating blood just as dissolution of erythrocytes can be produced by placental extracts. Furthermore, Marshall presents evidence that in certain ungulates the trophoblast can ingest not only hemoglobin but red blood cells as well, and states that iron-containing granules have been observed in all trophoblasts examined, including that of the early ovum of Peters. In addition he refers to the work of Bonet who observed on the surface of villi "perfect and damaged erythrocytes in all states of degeneration, clumping and dissolution." Recently Kropp (4), using microincineration methods, noted that the decidual and connective tissue cells contain more iron than either the amniotic or chorionic epithelium.

METHOD. The radio-active iron used in these studies was prepared by the method described by Wilson and Kamen (10) at the Radiation Laboratory of the University of California. A measured quantity of the iron solution in water or fruit juice diluent was administered orally to women at selected times prior to the termination of their pregnancy. Intervals of from less than an hour to several days elapsed between the time of administration of the iron and delivery because of the natural impossibility of predicting accurately the delivery time, save in those instances when therapeutic abortion or cesarean section was scheduled for a precise hour. At the time of delivery or operation a specimen of the fetal blood from the umbilical vein was collected in an oxalated hematocrit tube. A corresponding specimen of the maternal blood was obtained by venepuncture at the same time. Ten cubic centimeters of blood were ample for analysis.

The fetuses which were obtained intact by hysterotomy as the result of therapeutic abortion were dissected for determination of radio-active iron in the different tissues. No attempt was made to perfuse these tissues because of technical difficulties. Methods for preparation and purification of material for isotope estimation have been described elsewhere (3).

EXPERIMENTAL OBSERVATIONS AND DISCUSSION. In table 1 are listed the concentrations of blood cell labeled iron following feeding single doses of the radio-active iron to women at various stages of gestation and at variable lengths of time prior to termination of pregnancy. The dosage level of the isotopic iron covered a range of about 1 to 122 mgm., the amount given being governed largely by the quantity available at the time of feeding. In any event, in these studies we were more concerned with the ratios of the isotope in the blood of the mother and fetus and with the relative distribution of the isotope in any one fetus, rather than the total absorption and utilization.

Examination of table 1 indicates that some radio-active iron which had been fed the mother was recovered in every instance from the blood of the fetus. The fetuses ranged in age from three months to term. With but one exception (no. 7), for which no explanation is available, the isotope was recovered from the maternal circulation at the time of delivery. The data, however, do not reveal any correlation between dosage and recovery of the iron. Other factors must determine uptake of this element.

If it is true that absorption of iron is conditioned by its need, and the evi-

dence at hand bears this out, then one might well expect that a woman, whose physiology must adapt itself to the needs of reproductive function, will attach iron when this becomes available. In one respect, woman's metabolism of iron differs from that of man—she must replace the iron lost at menstruation. While this may not result in anemia, it might, nevertheless, reduce her iron reserve. Furthermore, the need for iron would appear to be particularly acute in the event of anemia in pregnancy. Additional absorption and utilization of newly proffered iron would be expected to occur, even in the absence of anemia during pregnancy, in order to provide for iron storage in the fetus. Such storage of iron is of major importance in the nutrition of the new born, inasmuch

TABLE 1

Radio iron in maternal and fetal red blood cells following ingestion by mother

CASE		RADIO IRON FED MOTHER	INTERVAL BETWEEN FEEDING AND DELIVERY	RADIO IRON IN RED BLOOD CELLS AT DELIVERY. PER CENT OF AMOUNT FED PER 100 ML.		GESTATION TIME
				Maternal	Fetal	
		mgm.				*months*
1	(P.F.)	53	5 days	0.015	0.082	3
2*	(J.F.)	122	2 days	0.046	0.37	4
	(J.F.)	122	2 days	0.046	0.51	4
3	(M.L.)	62	2 days	0.08	0.37	4½
4	(V.G.)	16	2 days	0.16	0.16	4
5	(J.T.)	122	36 hrs.	0.573	0.030	6½
6	(L.K.)	64	10 days	0.25	0.35	9
7	(M.H.)	42	5 days	0.0	0.2±	9
8	(M.R.)	42	4 days	0.16±	0.02±	9
9	(A.E.)	32	3 days	0.013±	0.036	9
10	(H.S.)	0.9	33 hrs.	0.11±	0.05±	9
11	(V.T.)	62	8 hrs.	0.024±	0.054±	9
12	(E.A.)	1.9	2 hrs.	0.035	0.037	8½
13	(M.C.)	14	110 min.	0.07±	0.05±	9
14	(S.B.)	20	40 min.	0.01±	Trace	9

* Twin pregnancy.

as the period during which milk is an adequate diet for the average new born infant is limited by the duration of the reserve of iron.

As a general rule, the fetal red blood cell hematocrit at term is greater than that of the mother. Table 2 bears this out. In early pregnancy, however, the reverse situation apparently prevails, as illustrated in cases 1 to 5 inclusive. Granting that our limited data permit only speculation, it is of interest nevertheless that an inverse relationship seems to exist between the hematocrit and the content of radio-active iron recoverable from the red blood cells. Thus in early pregnancy the fetal red blood cells took on proportionately much more radio-active iron than did the maternal red blood cells.

The significance of this observation is, however, qualified by the fact that in the majority of the cases reported the blood radio-iron measurements were

made earlier than four days after feeding, too early for the maximum synthesis of labeled hemoglobin to have occurred. Therefore, these ratios may also partially mirror the relative rates of hemoglobin synthesis in mother and fetus. Also, the red cell volume of the fetus increases during gestation so that equal increments of labeled red blood cells represent progressively decreasing ratios of labeled blood cells to total blood cells in the fetus.

Since depletion of iron reserves has been shown to be a major factor in governing the absorption of this element, it is not surprising that in late pregnancy the mother utilizes increasing amounts of iron made available through diet.

Patients (cases 1 to 5 inclusive, table 3) who for various reasons were subjected to therapeutic abortion were fed single doses of the isotopic iron. Imme-

TABLE 2

Comparison of the radio iron content of fetal and maternal red blood cells with the red blood cell hematocrits

CASE	RADIO IRON IN CELLS. PER CENT OF AMOUNT FED PER 100 ML. RATIO: FETAL/MATERNAL %	RED BLOOD CELL HEMATOCRIT. RATIO: FETAL/MATERNAL %	DURATION OF PREGNANCY
			months
1	5.5	0.71	3
2a*	8.0	0.83	4
2b	11.0	0.97	4
3	4.6	0.93	$4\frac{1}{2}$
4	1.0	1.38	4
5	0.05	1.15	$6\frac{1}{2}$
6	1.4	2.42	9
7		1.39	9
8		1.36	9
9	2.8	1.32	9
10	0.45	0.87	9
11	2.3	1.63	9
12	1.06	1.09	$8\frac{1}{2}$
13		1.91	9
14		1.49	9

* Cases 2a and 2b were twins.

diately following operation samples of the maternal blood and cord blood were taken. In the smaller fetuses it was not practicable to study distribution in the various tissues due to the size of the specimens. In such instances, the whole fetus or such discrete parts as were conveniently dissectable were ashed. No attempt was made to perfuse the tissues, and it is admitted that an undetermined amount of blood remained in the tissue. Inasmuch as there is a tendency to concentrate iron in the red cells when hematopoiesis is actively taking place, some not insignificant amounts of the isotope are probably ascribed to certain tissues, whereas they actually belong to the contained blood. Since values for the blood or red cell volumes in the fetus are not available, we were unable to estimate the fraction in the circulation. In each instance the fetal blood was obtained from the cord.

It is to be noted that as gestation progresses the total accumulation of the radio-active iron in the fetus increases. The series is too small to attempt to say at what stage the greatest rate of iron uptake occurs, but it is quite likely from inspection of the data that most of the uptake occurs during the second and third trimester.

Aside from the concentration of the isotope in the red cells, it is quite evident that the only other tissue which is consistently high in isotope is the liver. Since a large part of the hematopoietic tissue in the fetal organism is located

TABLE 3

Distribution of radio iron in fetal tissues after feeding radio iron to mother

CASE	1 (M.C.)	2 (P.F.)	3* (J.F.)	4* (J.F.)	5 (M.L.)	6 (V.G.)	7 (J.T.)
Age of fetus, mo	2	2½	4	4	4½	5	7
Radio iron fed mother, mgm	16	53	122	122	62	16	122
Interval between feeding and delivery, days	7	5	2	2	2	2	1
Radio iron in red blood cells. Per cent of amount fed per 100 ml.							
Maternal	0.043	0.016	0.046	0.046	0.077	0.16	0.57
Fetal		0.082	0.37	0.51	0.37	0.16	0.030
Radio iron. Per cent of amount fed recovered from tissues							
Liver		0.0036	0.015	0.014	0.046	0.024	0.0096
Spleen					0.0024		0.0017
Kidneys					0.002		0.0065
Gastro-enteric tract							0.0004
Thymus gland							0.0026
Heart							0.0019
Viscera (excluding above)		0.0008	0.0008	0.0009	0.012	0.0045	
Head, entire					0.004	0.53	
Residue of fetus excluding above named tissues	0.0002†	0.00056		0.198	0.0023	0.79	
Amniotic fluid		0.00013	0.006	0.0003		0.00	
Total (excluding red blood cells)	0.0002	0.0051	0.0218‡	0.035	0.0689	1.349	0.0227‡

* Cases 3 and 4 are twins.

† Entire fetus.

‡ Incomplete analysis.

here, this is not surprising. It is interesting, nevertheless, since this same organ is the main seat of iron deposition following iron feeding in the adult, even though hematopoiesis is most active in the bone marrow in the adult.

The speed with which the radio iron appears in the fetal circulation is clearly illustrated in table 4. Radio iron can be demonstrated in the plasma in as little time as 15 minutes following feeding to an iron depleted animal. Radio iron has been demonstrated in the red cells as hemoglobin in as little as 5 hours (6). However, in the present study, iron, following absorption, had to be transported in the maternal circulation to the placenta where through some mechanism it

finally reached the fetal circulation. Here we note that minute but measurable amounts of this isotope can be demonstrated in the fetal circulation in transport in the plasma in as little time as 40 minutes after feeding the mother. The three cases shown in table 4, it will be noted, received rather small doses of the isotope because under these conditions the highest percentage absorption takes place. It is also essential that the radio iron used have a high specific activity in order to permit one to detect the extremely small increments of circulating iron. The quantities of iron determined may be appreciated when one considers that the plasma radio iron level in case 1, table 4, is of the order of 4 micrograms per 100 ml. of fetal plasma, the amount of plasma actually being studied amounting to only 8 ml. In case 3, table 4, the amount of radio iron appearing in the red cells of the mother and infant was of the order of 1 microgram per 100 ml. of red cells, and here only about 3 ml. of cells were actually used in measurements. It is obvious that chemical methods could hardly be expected to show such minute changes regardless of the degree of accuracy attained.

TABLE 4

Showing rapidity of appearance of radio iron in fetal plasma

CASE	RADIO IRON FED MOTHER	RADIO IRON RECOVERED. PER CENT OF AMOUNT FED PER 100 ML. PLASMA		INTERVAL BETWEEN FEEDING AND DELIVERY
		Maternal	Fetal	
	mgm.			*min.*
1 (S.B.)	20	0.13	0.026	40
2 (M.C.)	14	0.28	0.18	110
3 (E.A.)	1.9	0.27	0.17	120

It seems quite improbable that the only route of transmission of iron from the mother to the fetus must be through the complicated chain of events expressed in most texts, *i.e.*, maternal plasma iron to red cell iron, passing of the latter to the placenta, destruction of the red cell with liberation of the contained hemoglobin iron, absorption, and finally elaboration of the iron into hemoglobin of the fetal blood cells. It seems doubtful if the radio iron would follow all these steps before it appears in the fetal plasma in as little time as 40 minutes following feeding of the mother. It seems more improbable that in two hours' time these many reactions plus hemoglobin formation occur along with appearance of isotope in the red cells of the fetus. It would appear, therefore, that at least some iron reaching the fetus does not have as its immediate precursor the iron contained in the hemoglobin of the maternal red blood cells.

SUMMARY

1. Radio-active iron when fed in single doses to women near termination of pregnancy appears rapidly in the fetal circulation, definitely measurable amounts being present in 40 minutes. The speed of transfer suggests that plasma rather than the red blood cell is the vehicle.

2. Distribution of radio iron in fetal tissues following feeding of the mother and subsequent therapeutic abortion shows a wide dissemination of the isotope with the greatest concentration in the red blood cells. Of other tissues studied, the liver contained the greatest quantity.

3. In early pregnancy the fetal hematocrit on comparison with that of the mother is low. At this time more radio-active iron is proportionately present in the fetal red cells following feeding of a single dose to the mother. The converse applies in late pregnancy. The relationship of iron uptake to the needs of the organism for iron is discussed.

REFERENCES

(1) BUNGE, G. Ztschr. f. Physiol. Chem. **17:** 63, 1893.
        Ibid. **17:** 78, 1893.
(2) CHARRIN, M. M. Compt. rend. Soc. de Biol. **51:** 338, 1898.
(3) HAHN, P. F., W. F. BALE, W. M. BALFOUR. This Journal **135:** 600, 1942.
(4) KROPP, B. Anat. Rec. **77:** 407, 1940.
(5) MARSHALL, F. H. A. The physiology of reproduction. London, Longmans, Green and Company, 1932.
(6) MILLER, L. L. AND P. F. HAHN. J. Biol. Chem. **134:** 585, 1940.
(7) PASTORE. Cited by H. J. STANDER.
(8) SCHOLTEN, R. AND J. VEIT. Ztschr. f. Geburtsch. u. Gynak. **49:** 210, 1903.
(9) STANDER, H. J. Williams' Obstetrics. New York, D. Appleton Co., 1941.
(10) WILSON, R. R. AND M. D. KAMEN. Physiol. Rev. **54:** 1031, 1938.

A COMPARISON OF THE PHYSIOLOGICAL EFFECTS OF DIHYDRO-TACHYSTEROL AND VITAMIN D IN THE RACHITIC AND NORMAL DOG

HAROLD E. HARRISON AND HELEN C. HARRISON

From the New York Hospital and the Department of Pediatrics, Cornell University Medical College

Holtz and Schreiber (1) first demonstrated that, from the products formed by the irradiation of ergosterol, a "calcinosis factor" could be separated which was not antirachitic but would produce a hypercalcemia when administered to normal or parathyroidectomized animals. Further studies indicated that the sterol most potent in elevating the serum calcium was dihydrotachysterol, which has been isolated and its chemical structure determined (2). Albright, Bloomberg, Drake and Sulkowitch (3) studied the physiological properties of dihydrotachysterol and vitamin D and suggested that they differ in their effects upon the rate of renal excretion of phosphate. Shohl and Farber (4) and Correll and Wise (5) have reported that the solution of dihydrotachysterol known as A.T. 10 has slight antirachitic activity in the rat and chick respectively.

We have previously studied the effect of vitamin D on the renal excretion of phosphate (6). Under standard conditions there is a maximal rate of tubular reabsorption of phosphate. Phosphate appears in the urine when the rate at which this ion is filtered through the glomeruli exceeds the maximum capacity of the tubules to reabsorb phosphate. In this respect the tubular reabsorption of phosphate is analogous to that of glucose and certain other organic solutes (7). Shannon and Fisher (8) have termed the maximum capacity of the tubules to reabsorb glucose the glucose Tm. The similar property with respect to phosphate may be called the phosphate Tm. The concentration of phosphate in the plasma at equilibrium shows a close correlation with the phosphate Tm. Vitamin D was found to increase the phosphate Tm, thus increasing the concentration of phosphate in the plasma, and this was thought to be one of the mechanisms of the antirachitic action of vitamin D. As a logical extension of this investigation, we undertook to determine the effect of dihydrotachysterol upon the rate of renal tubular reabsorption of phosphate.

EXPERIMENTAL PROCEDURE AND RESULTS. Female mongrel puppies, two months of age, were fed a modification of the low-calcium, vitamin D-free diet described by Morgan (9). The intakes of calcium and phosphorus were 135 and 365 mgm. per day, respectively. The diets were adequate for growth of the animals. After the dogs had received this diet for two to three months, definite evidences of vitamin D deficiency were present as indicated by decreased concentrations of both calcium and phosphorus in the serum and roentgenological evidence of rachitic changes in the bones. Repeated determinations were then made of the renal clearances of creatinine and phosphate following an intra-

venous injection of phosphate according to the technique previously described (6). From these data the maximum rate of reabsorption of phosphate by the renal tubules was calculated. Following a number of control observations, vitamin D_2 or dihydrotachysterol was administered and the effect on the phosphate Tm and on the concentrations of calcium and phosphorus in the serum studied. The vitamin D_2 was given as a solution of irradiated ergosterol in oil, assayed upon rats. The concentration of vitamin D_2 was calculated on the basis of 40,000 units per milligram of pure vitamin.[1] The dihydrotachysterol was supplied in the form of the commercial preparation formerly known as A.T. 10. This solution is said to contain 0.5 per cent sterol but its potency is equivalent to only 1.25 mgm. crystalline dihydrotachysterol per cubic centimeter of solution.

The experimental results are shown diagrammatically in figures 1 to 7. The concentrations of calcium and phosphorus in the serum, determined in the post-
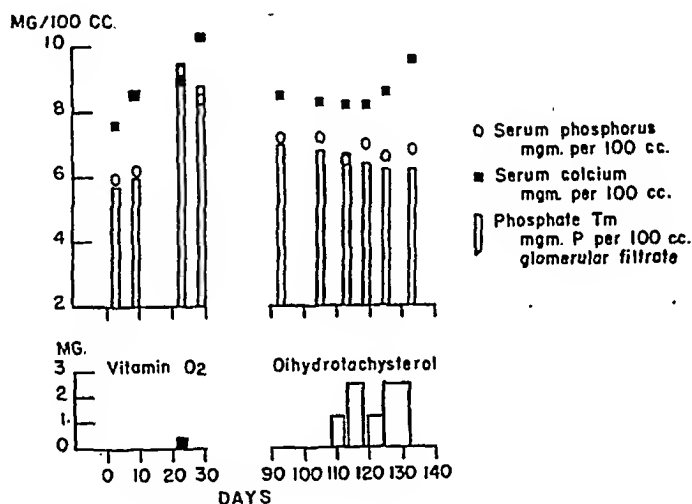
Fig. 1. Dog A—age 5 mos. Low calcium, vitamin D free diet

absorptive state, are shown by the solid squares and hollow circles respectively, both expressed as mgm. per 100 cc. serum. The phosphate Tm is indicated by the columns and is expressed as milligrams phosphorus reabsorbed per 100 cc. of glomerular filtrate formed. This value is equal to the so-called "equilibrium concentration," i.e., the concentration of phosphate in the serum at which the rate of filtration of phosphate through the glomeruli is equal to the maximum capacity of the tubules to reabsorb phosphate. The time interval in days is shown at the bottom of the figure, and the amounts of vitamin D and dihydrotachysterol administered are indicated as milligrams of active sterol per day.

In figure 1 are shown the results of the administration first of vitamin D and then of dihydrotachysterol to a rachitic dog. Over a period of three days 60,000 units of vitamin D, equivalent to 1.5 mgm. of active sterol, were given. There

[1] We are indebted to Dr. C. E. Bills of Mead Johnson and Co. who supplied the irradiated ergosterol used in these experiments.

was a prompt increase in phosphate Tm and a corresponding rise in the concentration of phosphate in the serum, as well as an increase in the concentration of calcium. No further vitamin D was given and in about 60 days the serum calcium was again decreased to 8 mgm. per 100 cc., and phosphate Tm and the serum phosphorus had fallen from the high levels found after the administration of vitamin D. Dihydrotachysterol was then given in doses of either 1.25 or 2.5 mgm. per day. During a period of three weeks approximately 44 mgm. of dihydrotachysterol were given with little effect upon the serum calcium except after prolonged treatment. The phosphate Tm and the concentration of phosphate in the serum remained unchanged.

In two other experiments, the results of which are shown in figures 2 and 3, dihydrotachysterol was given as soon as the dogs showed clear cut evidences of
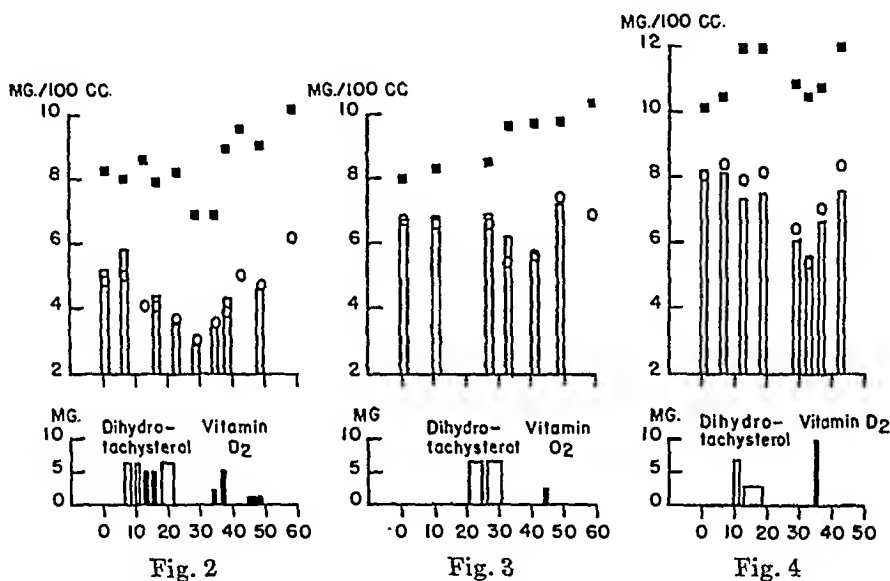
Fig. 2. Dog B—age 5 mos. Low calcium, vitamin D free diet
Fig. 3. Dog C—age 5 mos. Low calcium, vitamin D free diet
Fig. 4. Dog E—age 5 mos. Bread and meat diet

vitamin D deficiency. An amount of solution equivalent to 54 mgm. of dihydrotachysterol was given to dog B (fig. 2) without any demonstrable effect upon the serum calcium, which remained at about 8 mgm. per 100 cc. During and following the period of treatment the phosphate Tm and the concentration of serum phosphate dropped progressively, reaching a low level of about 3 mgm. per 100 cc. One week after the dihydrotachysterol treatment was stopped, the serum calcium fell to 6.8 mgm. per cent. The rapid drop of the serum calcium following the cessation of dihydrotachysterol therapy may indicate that this sterol did have a slight effect in preventing a decrease in the serum calcium during its use. Two weeks following the discontinuance of dihydrotachysterol treatment vitamin D was given. After the administration of 7 mgm., the concentration of calcium in the serum rose progressively as did the phosphate Tm and the serum phosphate. After a total of 10 mgm. of vitamin D had been

given the serum calcium had returned to the normal level of 10 mgm. per 100 cc. and the serum phosphate to 6 mgm. per 100 cc. In the case of dog C (fig. 3), the serum calcium remained low despite the administration of 6.25 mgm. of dihydrotachysterol daily for a period of 5 days. However, when treatment was continued for 5 more days, making a total dosage of 62.5 mgm., the serum calcium increased to 9.5 mgm. per 100 cc. At the end of this period of treatment the concentration of phosphate in the serum and the rate of renal tubular reabsorption of phosphate were lower than in the pretreatment studies. Seventeen days after the last dose of dihydrotachysterol had been given the dog received 2.5 mgm. of vitamin D. There was an immediate rise in the renal
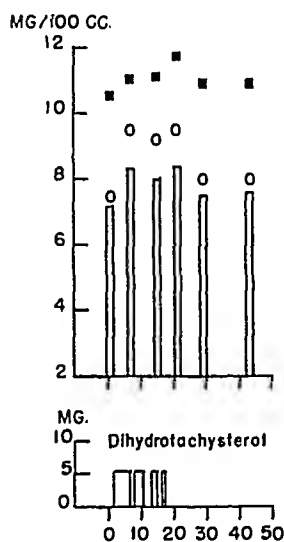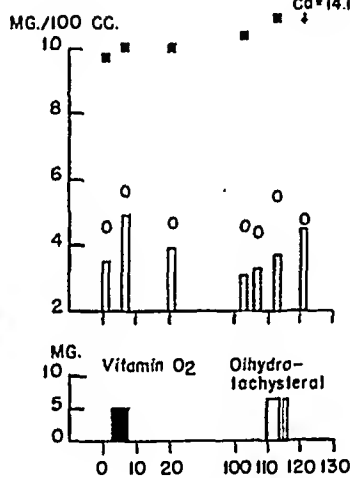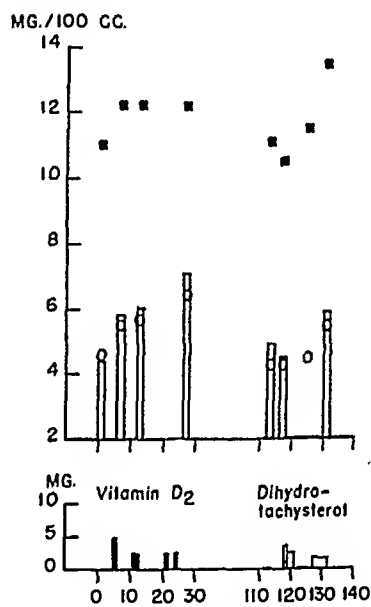
Fig. 5. Dog F—age 5 mos. Bread and meat diet supplemented with vitamin D
Fig. 6. Dog A—age 18 mos. Bread and meat diet
Fig. 7. Dog D—age 11 mos. Vitamin D free diet

tubular reabsorption of phosphate and in the concentration of phosphate in the serum. A further rise in the concentration of calcium in the serum also occurred.

In the next group of experiments dihydrotachysterol was administered to animals fed diets which contained vitamin D. Several young rapidly growing dogs were given a diet of white bread and ground lean beef. This diet supplied approximately the same amounts of calcium and phosphorus as the synthetic rachitogenic diet previously used. On this diet the dogs showed no evidence of vitamin D deficiency and the concentrations of calcium and phosphorus in the serum remained within the normal range. In figure 4 are shown the effects of dihydrotachysterol administered to a 5 month old dog who had been fed the bread and meat diet since the age of 2 months. Following the administration of 27.5 mgm. of dihydrotachysterol the concentration of calcium in the serum increased to almost 12 mgm. per 100 cc. but there was a slight decrease in the

rate of renal tubular reabsorption of phosphate. Following the cessation of dihydrotachysterol treatment the concentration of calcium in the serum returned to the pretreatment value in about 2 weeks, but there was a further decrease in the phosphate Tm with an associated decrease in the concentration of phosphate in the serum to 5.5 mgm. per 100 cc. At this time 10 mgm. of vitamin D were given and there was an immediate rise in phosphate Tm and in the concentration of phosphate in the serum. This would indicate that the bread and meat diet was not an adequate source of vitamin D.

In figure 5 are shown the results of another experiment on a young dog fed the bread and meat diet since the age of 2 months. In this experiment, however, 5000 I.U. of vitamin D were given daily starting 7 days before the administration of dihydrotachysterol. When dihydrotachysterol was given to this dog the renal tubular reabsorption of phosphate and the concentration of phosphate in the serum did not decrease, as in the previous experiment, but there was, on the contrary, a slight but definite increase which was maintained with continued administration of dihydrotachysterol. When the dihydrotachysterol was stopped, although the animal continued to receive 5000 I.U. of vitamin D daily, the phosphate Tm and the concentration of phosphate in the serum returned to the normal pretreatment levels.

In experiments on two other dogs the results indicate again that the phosphate Tm may be increased by the administration of dihydrotachysterol to dogs who have previously received vitamin D. In figure 6 are shown the results obtained in an adult dog fed a bread and meat diet. The administration of 20 mgm. of vitamin D resulted in the expected increase in phosphate Tm, although in this case no appreciable rise in the serum calcium was found. One hundred days later when dihydrotachysterol was given there was again a definite increase in the phosphate Tm and at this time a marked hypercalcemia. Similar findings for dog D are shown in figure 7. This animal was fed a synthetic vitamin D free diet. The administration of vitamin D was followed by a marked increase in the rate of tubular reabsorption of phosphate and also an increased concentration of calcium in the serum. Again, when dihydrotachysterol was given 100 days later, although no vitamin D had been given in the interim, there was an increase in the rate of tubular reabsorption of phosphate and a rise in the serum calcium. In these latter two experiments in the adult animal it is probable that sufficient amounts of vitamin D had been administered in the first series of studies so that the vitamin D stores of the body were still adequate 100 days later, at which time dihydrotachysterol was given. McChesney and Messer (10) have shown that in normal adult dogs receiving an adequate diet containing vitamin D the administration of dihydrotachysterol results in an increased concentration of phosphate as well as calcium in the serum, and the results are comparable with those obtained after the administration of large doses of vitamin D.

DISCUSSION. The experimental data indicate that the results of treatment with dihydrotachysterol are markedly different in the dog depleted of vitamin D from those in the dog who has had an adequate intake of this vitamin. Dihy-

drotachysterol cannot substitute for vitamin D in the rachitic dog, although it may have a vitamin D like effect in the normal dog.

When dihydrotachysterol is given to a dog who has received adequate amounts of vitamin D the phosphate Tm is increased, an effect comparable to that produced by an excess of vitamin D. However, in the rachitic dog the administration of dihydrotachysterol results either in no change or in an actual decrease in the phosphate Tm with a corresponding fall in the concentration of phosphate in the serum. This effect is noted even when enough dihydrotachysterol is given to produce a slight increase in the concentration of serum calcium. The phosphate Tm continues to decrease progressively following the cessation of dihydrotachysterol treatment until vitamin D is given, when there is the usual rise obtained with this vitamin. The decrease in renal tubular reabsorption of phosphate observed in the vitamin D-deficient animal following dihydrotachysterol administration suggests a parathyroid hormone-like effect (6). These results again indicate the importance of renal tubular reabsorption of phosphate in maintaining the normal phosphate economy of the body, and support the suggestion that the effect of vitamin D in increasing phosphate Tm is one of the mechanisms of its antirachitic effect.

The present experiments agree in part with Albright et al. (3) that one of the essential differences between the physiological effects of dihydrotachysterol and vitamin D is in their action upon the renal excretion of phosphate. However, there is also a striking difference between dihydrotachysterol and vitamin D in their effect upon the serum calcium in the rachitic dog. Dihydrotachysterol is relatively ineffective in increasing the concentration of calcium in the vitamin D depleted dog. This lack of potency of dihydrotachysterol in the absence of vitamin D has also been noted in human infants suffering from rickets and tetany. Harnapp (11) and Woo, Fan and Chu (12) have shown that the administration of solutions of dihydrotachysterol failed to raise satisfactorily the serum calcium of such infants, whereas preparations of vitamin D were immediately effective.

The experimental findings suggest that vitamin D is essential for certain cellular functions and cannot be replaced by dihydrotachysterol. If the vitamin D requirements of the tissues are satisfied, dihydrotachysterol can then supplement the essential vitamin and produce an effect similar to that of an excess of vitamin D.

The interrelationships between the parathyroid hormone and the effects of vitamin D and dihydrotachysterol are not as yet explained. In the subject with intact parathyroids, vitamin D increases the concentration of phosphate in the serum. However, in patients with hypoparathyroidism the abnormally high concentration of phosphate is decreased by the administration of both vitamin D (3, 13, 14) and dihydrotachysterol. This phase of the problem requires further study.

SUMMARY

The effects of dihydrotachysterol and vitamin D on the rate of reabsorption of phosphate by the renal tubules (phosphate Tm), and the concentrations of

phosphate and calcium in the serum were determined in rachitic and normal dogs. When vitamin D is given to the rachitic dog a prompt increase in phosphate Tm is found, associated with a rise in the concentrations of phosphate and calcium in the serum. Following the administration of dihydrotachysterol to the vitamin D depleted dog there is a progressive decrease in phosphate Tm and in the serum phosphate. The concentration of calcium in the serum may be slightly increased if large amounts of dihydrotachysterol are given. In the normal dog, however, which has been given vitamin D, dihydrotachysterol produces a marked elevation of the serum calcium and also an increase in phosphate Tm and the concentration of phosphate in the serum. The results indicate that although the physiological effects of dihydrotachysterol in the normal dog are similar to those of vitamin D, dihydrotachysterol can not substitute for vitamin D in the rachitic dog.

REFERENCES

(1) HOLTZ, F. AND E. SCHREIBER. Ztschr. f. Physiol. Chem. 191: 1, 1930.
(2) VON WERDER, F. Ztschr. f. Physiol. Chem. 260: 119, 1939.
(3) ALBRIGHT, F., E. BLOOMBERG, T. DRAKE AND H. W. SULKOWITCH. J. Clin. Investigation 17: 317, 1938.
(4) SHOHL, A. T. AND S. FARBER. J. Nutrition 21: 147, 1941.
(5) CORRELL, J. T. AND E. C. WISE. J. Nutrition 23: 217, 1942.
(6) HARRISON, H. E. AND H. C. HARRISON. J. Clin. Investigation 20: 47, 1941.
(7) SHANNON, J. A. Physiol. Rev. 19: 63, 1939.
(8) SHANNON, J. A. AND S. FISHER. This Journal 122: 765, 1938.
(9) MORGAN, A. F. Univ. of Calif. Publications in Physiology 8: 61, 1934.
(10) McCHESNEY, E. W. AND F. MESSER. This Journal 135: 577, 1941–42.
(11) HARNAPP, G. O. Monatschr. f. Kinderheilkunde 63: 262, 1935.
(12) WOO, T. T., C. FAN AND F. T. CHU. Chinese Med. J. 60: 99, 1941.
(13) ROSE, E., W. H. PERLOFF AND F. W. SUNDERMAN. Am. J. Med. Sci. 202: 691, 1941.
(14) KLATSKIN, G. J. Clin. Investigation 17: 431, 1938.

ON THE THROMBOPLASTIC ACTIVITY OF BRAIN AND SKIN EXTRACTS

ALFRED LEWIN COPLEY[1]

From the Hixon Laboratory for Medical Research, University of Kansas, School of Medicine, Kansas City

Variations of thromboplastic activity may be estimated by comparing the coagulation time on one sample of plasma. The thromboplastic substances used in these comparisons may be prepared from different organs of various species. Quick (1) developed the prothrombin time test by determining the coagulation time of recalcified plasma to which an excess of brain thromboplastin is added. He showed that the prothrombin time may vary depending on the origin of the thromboplastin and the species in which it was used (2), and suggested that seasonal variations might influence the thromboplastic activity of tissues (3). The purpose of this study was to determine if the age of the animal would influence the thromboplastic activity of their brains and if the aging of chicken plasma at room temperature would have an effect on the prothrombin time. Furthermore, we wished to determine if there is any correlation between the prothrombin time and the coagulation time of recalcified oxalated plasma. Since the activity of skin thromboplastin has not been examined, we prepared thromboplastic substances from skin and brain of rabbits and chickens for purposes of comparison. In the course of this study it became apparent that the widely accepted explanation of the phenomenon of prothrombin time which is based on the Morawitz concept of the classical theory of blood coagulation (4) could not be correlated with our findings. We therefore propose a tentative conception which is principally based on Howell's theory (4).

METHODS. The plasma was decalcified as described by Quick (1) by adding 1 volume of 0.1 M sodium oxalate solution to 9 volumes of blood. Blood was drawn from the antebrachial vein of humans, the jugular vein of chickens and dogs, and the heart of rabbits and immediately mixed with sodium oxalate solution. The oxalated blood was centrifuged at 1500 r.p.m. for 10 minutes and the plasma drawn off. For the plasma coagulation times, with or without the addition of thromboplastin emulsion, 0.1 cc. of 0.025 M calcium chloride solution was used. The coagulation time of recalcified plasma was determined by adding 0.1 cc. of 0.9 per cent sodium chloride solution to 0.1 cc. decalcified plasma. All tests were run in duplicate and the appearance of a gel was used as the endpoint for the plasma coagulation time.

Thromboplastic substances of brain were prepared with acetone washings according to Quick's method (1) and by a drying method similar to the procedure of Link et al. (5). Quick's method was applied to experiments listed in tables 1, 2 and 3. Chicken skin thromboplastin for experiments compiled in table 3 was prepared by mincing (without sand) and extracting the minced material

with acetone. One hundred milligrams of each dry thromboplastic substance was mixed with 5.0 cc. of 0.9 per cent sodium chloride solution and incubated for

TABLE 1

Prothrombin time of human and dog plasma with brain thromboplastin from rabbits of different ages

BRAIN THROMBOPLASTIN AGE OF RABBIT	PLASMA PROTHROMBIN TIME IN SECONDS OF			
	Human I	Human II	Dog I	Dog II
3 weeks	24	24	10	8
6 weeks	23	23	9	7
3 months	19	19	9	7
4 months	18	20	8	6
10 months	14	16	7	8
15 months	16	17	7	6

TABLE 2

Effect of aging at room temperature (26°C.) upon the prothrombin time of chicken plasma with chicken brain thromboplastin

PLASMA	PROTHROMBIN TIME IN SECONDS			
	Aging of plasma:			
	1 hour	3 hours	8 hours	24 hours
Chicken A..............	28	42	125	>3600
Chicken B..............	33	44	90	>3600
Chicken C.	38	42	106	>3600
Chicken D..............	36	41	65	>3600

TABLE 3

Prothrombin time in chicken plasma obtained with different thromboplastins and their activity expressed by the thromboplastin coefficient

CHICKEN NUMBER	PROTHROMBIN TIME IN SECONDS WITH THROMBOPLASTIN OF			COAGULATION TIME OF RECALCIFIED PLASMA	THROMBOPLASTIN COEFFICIENT WITH THROMBOPLASTIN OF		
	Chicken brain	Chicken skin	Rabbit brain		Chicken brain	Chicken skin	Rabbit brain
				seconds			
1	23	180	180	290	12.6	1.6	1.6
2	23	360	270	600	26.1	1.7	2.2
3	22	210	240	720	32.7	3.4	3.0
4	25	300	510	1410	56.4	4.7	2.8
5	28	240	240	540	19.3	2.3	2.3
6	20	330	540	1020	51.0	3.1	1.9
7	32	420	600	3120	97.5	7.4	5.2
8	28	360	420	750	26.8	2.1	1.8
9	23	240	240	960	41.7	4.0	4.0

15 minutes at 50 to 55°C. For the experiments in table 4, the brain and skin of rabbits and chickens were minced in a mortar within 2 hours after slaughtering.

The cutaneous tissues were scraped from the inside of the rabbit's pelt. One portion of each organ was treated with acetone and an equal portion was prepared without acetone. The minced tissues were dried for 24 hours at 36 to 38°C. in a desiccator containing calcium chloride. The dry material was weighed in 250 mgm. lots and each preparation was subjected to further grinding with sea sand (Merck). The brains were also ground in sand in order to obtain thromboplastin preparations under similar conditions. The disintegrated material was taken up with 12.5 cc. of 0.9 per cent sodium chloride solution and centrifuged at a constant speed of 1000 r.p.m. for 5 minutes. The supernatant fluid was decanted and incubated in a water bath at 55°C. for 15 minutes. Equal volumes of 0.025 M calcium chloride solution and thromboplastin emulsion were mixed and 0.2 cc. of this mixture was added to 0.1 cc. of plasma.

RESULTS. Table 1 demonstrates the thromboplastic activity on the prothrombin time of human and dog plasma of brains from rabbits varying in age from 3 weeks to 15 months. It appears that brain thromboplastins from young and

TABLE 4

The accelerating effect of thromboplastins from brain and skin of rabbit and chicken on the coagulation time of recalcified plasma expressed by the thromboplastin coefficient

PLASMA	THROMBOPLASTIN COEFFICIENT							
	Rabbit brain		Rabbit skin		Chicken brain		Chicken skin	
	A	D	A	D	A	D	A	D
Dog I.....................	6.6	8.0	3.8	3.2	2.9	2.2	2.6	2.4
Dog II.....................	4.3	5.6	2.9	2.3	1.9	1.5	1.6	1.6
Rabbit I..................	6.5	5.3	2.1	2.0	1.2	0.9	0.8	0.9
Rabbit II..................	7.1	6.9	4.5	2.5	2.2	1.6	1.7	1.7
Chicken I..................	4.4	6.6	8.7	5.1	133	123	25	75
Chicken II..................	7.5	7.0	9.4	6.0	125	113	38	93
Human I...................	6.5	8.2	5.4	4.0	1.0	0.7	1.1	1.0
Human II.................	6.0	8.0	4.2	3.9	1.1	1.0	1.1	1.2

A = prepared with acetone; D = prepared without acetone.

adult rabbits possess similar thromboplastic activity. However, there is a slight prolongation of prothrombin time with brain thromboplastins of 3 and 6 week old rabbits.

Table 2 shows that the prothrombin time of chicken plasma which had been allowed to stand at room temperature for 8 hours was from 1.8 to 4.5 times longer than after standing for 1 hour. Further, after aging the plasma for 24 hours, the prothrombin time exceeded 1 hour.

Instead of expressing the accelerating effect of the thromboplastin emulsion upon the oxalated plasma coagulation time in seconds as is usually done, we express this thromboplastic activity as a ratio of the coagulation time of recalcified plasma to the prothrombin time. This coefficient of thromboplastic activity equals 1 if the added thromboplastin emulsion does not exhibit any activity; whereas, when this coefficient is greater than 1 it expresses the relative activity of a given thromboplastin emulsion upon the coagulation time of the same plasma.

In table 3 the prothrombin time in chicken plasma obtained with thromboplastins of chicken brain, chicken skin, and rabbit brain are compared with the coagulation time of the recalcified plasma in seconds. The activity of the three thromboplastic substances is also compared with their corresponding coefficient of thromboplastic activity. For convenience we have named the latter "thromboplastin coefficient." Chicken brain thromboplastic activity, as expressed by the prothrombin time, varied between 22 and 32 seconds. Prothrombin times with chicken skin and rabbit brain were markedly prolonged, and it would appear that these thromboplastins have only insignificant activities. However, when these values are compared with the coagulation time of recalcified plasma, one observes that both preparations did shorten the coagulation time. Even though the prothrombin times fell in a narrow range, the acceleration of coagulation by the addition of thromboplastin emulsion varied markedly. Chicken brain in chicken plasma was much more active in accelerating the coagulation than either chicken skin or rabbit brain thromboplastin.

Table 4 exhibits the accelerating effect of thromboplastin obtained from brain and skin of rabbits and chickens on the plasma of dogs, rabbits, chickens and humans. The acceleration with two kinds of thromboplastin prepared from each organ with and without acetone was expressed by the thromboplastin coefficient. Rabbit brain was most active in dog plasma. Chicken skin and brain showed approximately equal activity. The rabbit skin, however, was more active than either chicken preparation. Rabbit brain thromboplastin was more active than rabbit skin in rabbit plasma. Chicken brain and skin thromboplastins showed slight activity in rabbit II plasma and no activity in rabbit I plasma. In chicken plasma, chicken brain was stronger than chicken skin. Rabbit brain and skin showed slight activity by comparison to chicken thromboplastin; however, activity was definitely present. In human plasma, rabbit brain was stronger than rabbit skin. Neither preparation of chicken thromboplastin exhibited any significant acceleration in human plasma. Acetone (A) and dry (D) preparations of thromboplastin showed variation of activity in the different plasmas.

DISCUSSION. We employed 20 mgm. per cubic centimeter of thromboplastic substance in all the studies, because 20 to 60 mgm. rabbit brain thromboplastin per cubic centimeter exhibited similar prothrombin times in human and dog plasma. It appears that the age of the rabbit up to 6 weeks has only slight effect on the thromboplastic activity of the brain upon human plasma. The brains of all rabbits over 3 months show essentially the same activity. There is some controversy in the literature with regard to the prolongation of the prothrombin time due to storage of blood or plasma. According to Rhoads and Panzer (6) who used a modification of Quick's method, this prolongation occurred after a few days. Similar observations were made by Quick (7). Warner et al. (8), on the contrary, who used Quick's method and the 2-stage method of Smith, Warner and Brinkhous (9), did not find such a marked prolongation within so short a time. Nevertheless, chicken plasma kept at room temperature shows a tendency to prolong prothrombin times with extreme rapidity. It is of interest in this connection that oxalated goose blood has been found by Quick (7) to prolong, upon

standing, the prothrombin time even more rapidly than human blood. From this it might be inferred that avian oxalated blood inhibits, on storage, thromboplastic activity to a greater extent than oxalated blood of mammals. An explanation for this phenomenon can not be given at the present time. It may be noted that upon standing the coagulation time of recalcified chicken plasma also rapidly became longer. The finding of Tocantins (10) that loss of CO_2 prolongs the prothrombin time may not be the entire explanation. It appears that, when using the same thromboplastic substance, temperature, sterile conditions and the genus or even species, from which the plasma was obtained, influence the prothrombin time in aging plasma.

Quick (2) studied the prothrombin time in various species using various thromboplastins. He concluded that certain thromboplastins show their greatest activity when reacting with their own prothrombins. This, however, does not seem to be always true since he found in human plasma a significantly shorter prothrombin time with rabbit brain than with human brain. Warner, Brinkhous and Smith (11) studied the thrombin titers in various vertebrates with their method, and found them far higher in mammals than in the lower vertebrates and that the values rose progressively according to the position in the evolutionary scale. They have tested many organs as a source of thromboplastin and found that thromboplastin prepared from brain or lung was most active. In most cases, however, they have observed that organ extracts from one species are almost equally effective in activating thrombin of the other species studied and that an element of species specificity was apparent with chicken plasma. We believe that apparently there is a trend towards specificity, but we do not believe that this presents the full explanation for the various activities of different thromboplastins.

The coagulation time of recalcified plasma may exhibit the activity of the thromboplastic substance which, due to platelet disintegration or from other sources, is originally present in the plasma. The "thromboplastin coefficient" was introduced to express the ratio of the initial thromboplastic activity of the plasma to the activity of thromboplastin in excess. We believe that using seconds alone as a measure of thromboplastic activity in different plasmas is erroneous. For example, if one studies dog plasma with a prothrombin time of 10 seconds and human plasma with a prothrombin time of 23 seconds, one may assume that in dog plasma there is a 2.3 times greater thromboplastic activity than in human plasma. However, this apparently is not true, because, upon recalcification, the same human plasma clots in 360 seconds and the same dog plasma, in 160 seconds. The thromboplastic activity as determined by the thromboplastin coefficient can thus be the same, even though the prothrombin times in seconds in both instances do not agree. The thromboplastin coefficient may not vary greatly. This is due either to the short recalcified plasma coagulation time or to the proportionate increases both in the latter and in the prothrombin time. The vast difference in the thromboplastin coefficient which occurred with the chicken brain thromboplastin is not attributable to the prothrombin time, as can be seen from table 3, but to wide variations of the recalcified plasma coagulation time. As endpoints of the prothrombin time, or recal-

cified plasma coagulation time, we always used the appearance of a gel. For this reason the recalcified plasma coagulation time is probably longer than may be expected. It may be noted that precipitation could be observed before gelation had started. Precipitation of fibrin needles was observed to occur mostly simultaneously with gel formation in plasma coagulation. However in hemophilic plasma or blood, precipitates of fibrin or little clots often occur long before gelation. To our knowledge there are no special studies in the literature regarding the occurrence and relationships of both phenomena.

Thromboplastic acceleration of coagulation time in plasma of the same, or different, species cannot be explained satisfactorily by the concept of Quick (2). According to him, the prothrombin time expressed in seconds indicates the concentration of prothrombin which is present in the plasma. This becomes further evident when thromboplastins from different organs of the same species are examined. Furthermore, no explanation is offered by the Quick concept for the discrepancies which exist between prothrombin time and coagulation time of recalcified plasma. Therefore, we offer a tentative explanation which is based upon recent findings of other authors which hitherto were not sufficiently correlated to explain the discrepancies in our results.

The contention that prothrombin is a precursor of thrombin which seems to be generally accepted today is again a matter of controversy. Dyckerhoff et al. (12, 13) have recently stated that thromboplastin (thrombokinase) activates thrombin in a similar manner as enterokinase activates trypsin by the exclusion of inhibitory substances. They offered this theory as a reasonable explanation for the many observations in the literature which are not completely explainable by the Morawitz theory. They suggest that completely formed thrombin is present in the circulation, and that its action is hindered by inhibiting substances. In Howell's theory, heparin in the circulating blood is combined to form a prothrombin-antiprothrombin complex. Thromboplastin in shed blood combines with the heparin, freeing the prothrombin which is then activated to thrombin by the calcium of the blood.

Deducing from both theories, our view is represented in the following schema:

Thromboplastin

Prothrombin complex ← — — — —Ca^{++}

Heparin — Complementary factor — Inactive thrombin

Antithrombin
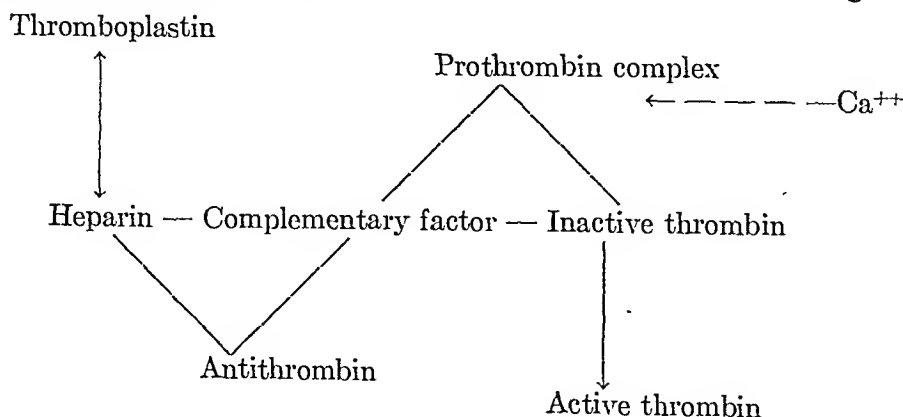
Active thrombin

where, ⟶ interaction between
← — — — activation influence on
⟶ resulting in

The complementary factor plus thrombin constitutes the prothrombin complex. According to this view, prothrombin, contrary to the theory of Morawitz, is only a precursor of thrombin in that in the heparin-prothrombin complex, preformed thrombin is merely inactivated by antithrombin which is composed of heparin and the complementary factor. Thromboplastin in the presence of calcium and the complementary factor has its neutralizing effect upon heparin, thus activating the thrombin. Howell and Holt (14) have already shown that heparin acts as an antithrombin only in the presence of proantithrombin which is a thermolabile antecedent substance in plasma or serum. This substance was demonstrated by Quick (15) to be contained in albumin. Recently Jaques and Mustard (16) and Ziff and Chargaff (17) have found this factor in the albumin fraction, but not as crystalline serum albumin. Contemporaneously with these authors it was found by Stewart and Rourke (18) that in a particular fraction of plasma albumin the property of inactivating thrombin is resident. Howell's contention that heparin has a direct inhibitory action in the first phase of coagulation (anti- prothrombin) was recently substantiated by Brinkhous, Smith, Warner and Seegers (19). The findings of these authors demonstrate that, furthermore, plasma contains a first phase complementary factor which is essential for the antiprothrombin effect of heparin. This conclusion was verified by Astrup (20). Brinkhous et al. offer the hypothesis that the complementary factor is identical to the proantithrombin of Howell and also serves in the first phase of coagulation in combination with heparin as antiprothrombin or antithromboplastin. From these recent researches, although definite evidence is lacking, it may be assumed that the antithrombin in the thrombin activation phase and the fibrinogen conversion phase are identical, namely, heparin plus complementary factor. On the other hand, the hypothesis that the prothrombin complex consists of complementary factor and inactive thrombin has, at present, no experimental basis. It is of interest, however, that thrombin was activated from prothrombin by dialysis and on standing in solution (21), by certain proteolytically active snake poisons (22) and by trypsin (23). The classical theory expresses the specificity of thrombin action on the conversion of fibrinogen to fibrin (4, 24). There are several thrombin theories and we believe, with Howell, that in their development the solution of the problem is to be expected.

On this basis we feel that the prothrombin time expresses the speed with which the activation of thrombin occurs, and the degree of concentration of the activated thrombin from the prothrombin complex. From this it might be inferred that the prothrombin time does not necessarily determine quantitatively the prothrombin content. Therefore it was preferred to use the current term "prothrombin time" instead of prothrombin concentration for the test. Ferguson (25) has demonstrated that inhibitors of the nature of antiprothrombin and proantithrombin influence the results obtained by the prothrombin time methods. He concluded that certain clinical anomalies as expressed by the prothrombin time may reside in the associated inhibitor variables and not in the "prothrombin per se". It should be pointed out that Quick (7) stated that his test and the method of Smith et al. "are based on unproved assumption; therefore the validity

of the results which they yield can be judged only by correlation with clincial observation". We believe that different preparations of thromboplastin may activate thrombin to varying degrees and rates when combining with the heparin of the heparin-prothrombin complex.

SUMMARY

1. The age of the rabbit up to six weeks has only slight influence upon the thromboplastic activity of their brains upon human plasma. Over three months the age has no significant effect.

2. In chicken plasma the prothrombin time is rapidly increased upon standing at room temperature.

3. The ratio of coagulation time of recalcified oxalated plasma to the prothrombin time was named "thromboplastin coefficient". It was used to estimate the activity of a given thromboplastic substance.

4. Rabbit skin and chicken skin exhibit thromboplastic activities to various degrees.

5. Chicken brain and chicken skin have no activity with human plasma and most activity with chicken plasma.

6. The trend of apparent species specificity of thromboplastin is discussed.

7. The marked discrepancy in prothrombin time of the same plasma due to various thromboplastins cannot be explained by the Morawitz theory of blood coagulation. A concept which best explains this discrepancy is advanced. This concept is mainly based on Howell's theory and recent researches of other authors.

REFERENCES

(1) Quick, A. J. J. A. M. A. **110**: 1658, 1938.
(2) Quick, A. J. This Journal **132**: 239, 1941.
(3) Quick, A. J. This Journal **114**: 282, 1936.
(4) Howell, W. H. Physiol. Rev. **15**: 435, 1935.
(5) Campbell, H. A., W. K. Smith, W. L. Roberts and K. P. Link. J. Biol. Chem. **138**: 1, 1941.
(6) Rhoads, J. E. and L. M. Panzer. J. A. M. A. **112**: 309, 1939.
(7) Quick, A. J. J. A. M. A. **114**: 1342, 1940.
(8) Warner, E. D., E. L. DeGowin and W. H. Seegers. Proc. Soc. Exper. Biol. and Med. **43**: 251, 1940.
(9) Smith, H. P., E. D. Warner and K. M. Brinkhous. J. Exper. Med. **66**: 801, 1937.
(10) Tocantins, L. M. Proc. Soc. Exper. Biol. and Med. **49**: 251, 1942.
(11) Warner, E. D., K. M. Brinkhous and H. P. Smith. This Journal **135**: 296, 1939.
(12) Dyckerhoff, H., H. Miehler and R. Steiner. Biochem. Ztschr. **297**: 342, 1938.
(13) Dyckerhoff, H. Biochem. Ztschr. **305**: 181, 1940.
(14) Howell, W. H. and E. Holt. This Journal **47**: 328, 1918.
(15) Quick, A. J. This Journal **123**: 712, 1938.
(16) Jacques, L. B. and R. A. Mustard. Biochem. J. **34**: 153, 1940.
(17) Ziff, M. and E. Chargaff. J. Biol. Chem. **136**: 689, 1940.

(18) STEWART, J. D. AND C. M. ROURKE. J. Clin. Investigation **19**: 695, 1940.
(19) BRINKHOUS, K. M., H. P. SMITH, E. D. WARNER AND W. H. SEEGERS. This Journal **125**: 683, 1939.
(20) ASTRUP, T. Science **90**: 36, 1939.
(21) CEKADA, E. B. This Journal **78**: 512, 1926.
(22) EAGLE, H. J. Exper. Med. **65**: 613, 1937.
(23) EAGLE, H. J. Gen. Physiol. **20**: 543, 1937.
(24) COPLEY, A. L. This Journal **126**: 310, 1939.
(25) FERGUSON, J. H. Proc. Soc. Exper. Biol. and Med. **47**: 538, 1941.

A QUANTITATIVE MEASURE OF THE VASOMOTOR TONE IN THE HINDLIMB MUSCLES OF THE DOG

JOHN R. PAPPENHEIMER AND JULIAN P. MAES

From the Department of Physiology, College of Physicians and Surgeons, Columbia University

A change of blood flow through any particular part of the vascular bed may be the result of a change in any one of the factors found by Poiseuille (1844) to determine the rate of flow of fluids through rigid tubes. These factors include the pressure, the dimensions of the tube, and the viscosity of the fluid. The vasomotor nerves act on the circulation by altering the dimensions of the blood vessels; their effects on the blood flow may be complicated, however, by simultaneous changes of blood pressure or by the liberation of substances into the circulation which themselves alter the dimensions of the blood vessels. Another factor, not previously investigated, complicates the interpretation of changes in blood flow. It will be shown in this paper that the effects of the vasomotor nerves are in large part the result of changes in the internal resistance (apparent viscosity) of the blood itself.

The difficulties of separately evaluating the effects of each of these variables are largely responsible for the present lack of quantitative information about the part played by the vasomotor nerves in the control of the peripheral circulation. These difficulties have been overcome in the pump-lung innervated hindlimb preparation of the dog. The hindlimb muscles of anesthetized dogs are perfused with defibrinated blood from a pump-lung circulation while connections with the central nervous system are retained through the sciatic nerve. The pressure and the composition of the blood supplying the muscles are thus made independent of changes in the animal. The effects of the vasomotor nerves on the circulation can be studied quantitatively by reversibly blocking the nerve and recording the changes of blood flow. Finally the effects of the nerves on the apparent viscosity of the blood can be detected and measured by the methods described below.

I. METHODS. 1. *Perfusion.* The apparatus and technique of perfusion were similar to those used by Eggleton, Pappenheimer and Winton (1940) for the perfusion of the isolated kidney. In this form of apparatus the perfusion pressure may be adjusted to any constant value which is essentially independent of the resistance of the perfused organ. The experiments involving perfusion with Ringer's solution necessitated a second artificial circulation which was connected with the pump-lung circuit in such a way that either blood or Ringer's solution could be used to supply the limb. The composition of the Ringer's solution was NaCl 0.9 per cent, KCl 0.042 per cent, CaCl₂ 0.024 per cent and NaHCO₃ 0.03 per cent.

2. *Pressure.* The perfusion pressure was measured with a mercury manom-

eter. It was corrected for the difference in height between the manometer and the artery and for the pressure fall across the arterial perfusion cannula at any particular flow. The latter correction was obtained for each cannula by suitable calibrations on blood and on Ringer's solution.

3. *Blood flow.* The venous outflow was measured with a stopwatch and measuring cylinder. It was also recorded continuously by the method of Gaddum (1929).

4. *Pressure-flow curves.* The perfusion pressure was varied in steps and the flow corresponding to each pressure measured as soon as the flow had reached a steady value as indicated by the continuous record (fig. 1). Each measurement required about two minutes. The results were then plotted against time and the pressure-flow curve at any particular time was obtained by interpolation.

5. *Oxygen saturation.* The oxygen saturation was measured and recorded continuously by the methods of Kramer and Winton (1939) with the modifications described by Pappenheimer (1941).

6. *Infusion of adrenalin.* Adrenalin was made up in saline and the solution evacuated in the Van Slyke apparatus. It was kept under oil in a capillary influsion apparatus which delivered 0.10 to 1.00 cc./min. within an error of about ±0.01 cc./min.

7. *Cooling the nerve.* The sciatic nerve was encircled with a nickel-plated copper tube. The nerve was protected from direct contact with the metal by cotton moistened with saline. The tube could be connected with reservoirs containing ice and brine or warm water. Thermometers were inserted near the inflow and outflow and the temperature of the nerve controlled by varying the rate of flow of fluid in the tube.

8. *The innervated perfused hindlimb preparation.* A dog weighing 10 to 20 kgm. was anesthetized with nembutal or with chloralose. The tendons of the thigh muscles, the insertions of which lie around and below the knee-joint, were sectioned between ligatures and the articulation opened below the patella. The articular arteries and veins and the saphenous artery and vein were divided. A circular skin incision was made around the lower part of the calf. The small saphenous vein (which receives veins from the gastrocnemius muscle before it joins the posterior femoral vein) and the posterior femoral artery and vein were isolated and all their branches to the thigh muscles tied. A window was made in the femur with a circular saw, some of the marrow removed and the cavity packed with cotton. The sciatic nerve was dissected free of fat and the femoral artery and vein prepared for the perfusion cannulae. The blood supply of the tissues below the knee was in this way confined to the femoral artery.

During the first operation a second dog was anesthetized and the pump-lung preparation begun. The two operations were so timed that the limb was ready for perfusion one hour after starting the pump-lung circulation thus allowing time for "detoxication" of the blood by the lungs.

Excluding bone, about two-thirds of the tissues below the knee are muscle. In a typical preparation the skin weighed 51 grams, muscles 121 grams, and bone 179 grams. Only a small part of the skin appears to be perfused. Calculated

on the assumption that all the perfused tissue was muscle, the oxygen consumption was approximately 0.01 cc./gram/min. which is only slightly greater than the values obtained if special precautions are taken to confine the circulation to muscle (Pappenheimer, 1941). After some hours of perfusion a small leak may develop from the vessels around the cut surfaces of the skin. In our earlier experiments this leak was measured and recorded with a drop counter; it rarely
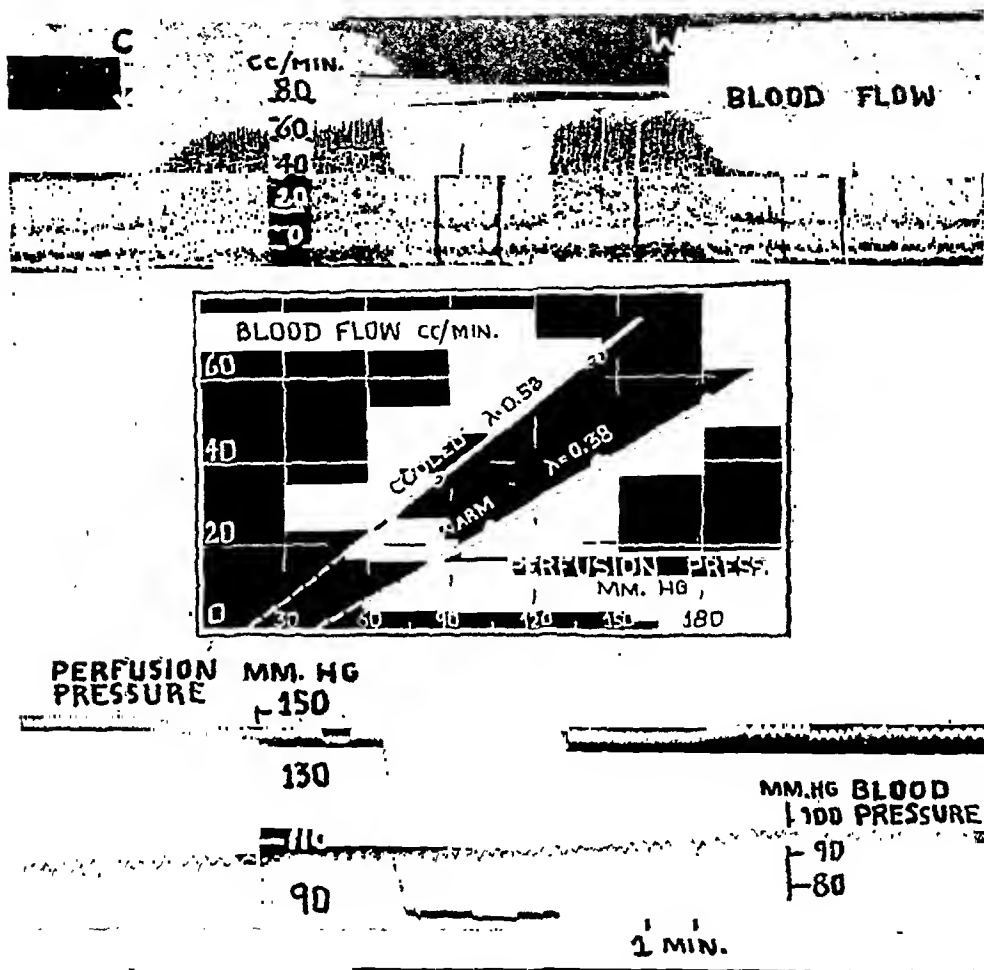
Fig. 1. The effects of cooling the sciatic nerve on the blood flow in the innervated perfused hindlimb muscles. Dog, 14 kgm. Nembutal. At C nerve cooled, at W nerve warmed. Pressure-flow data with nerve warm obtained before and after cooling. Note 1, reversibility of effects of cooling; 2, the effects of vasoconstriction on the slope and intercept of the pressure-flow curve.

amounted to more than 2 per cent of the total blood flow. The experiments were generally terminated by edema of the lungs in the perfusion circuit 5 to 9 hours after the perfusion was begun.

II. THE EFFECTS OF COOLING THE NERVE. Figure 1 illustrates the effects on the blood flow of cooling the nerve for a period of twelve minutes. It is seen that the blood flow measured at a pressure of 143 mm. Hg increased from 38 cc./min.

to 64 cc./min. where it remained constant. Pressure-flow data were obtained and the nerve was then warmed. Complete recovery of the nerve after these procedures is indicated by the return of the blood flow to 36 cc./min. at a pressure of 144 mm. Hg. In 61 similar experiments the nerve failed to recover twice although in a few instances recovery did not occur for several minutes. In five experiments in which the nerve was cut during a period of cooling no further changes of blood flow were observed. Curarization of the muscles had no detectable effect on the change of blood flow when the nerve was blocked.

It might be supposed that the change in blood flow at constant pressure which occurs when the nerve is blocked would be a quantitative measure of the degree of vasomotor activity. This is not in fact the case. It was found that for any constant degree of vasomotor tone the ratio of the blood flow with the nerve blocked to the flow with the nerve active varied greatly with the absolute value of the perfusion pressure (fig. 5). The reasons for this variation become clear when the effects of vasoconstriction on the relation between the pressure and the flow of blood are examined.
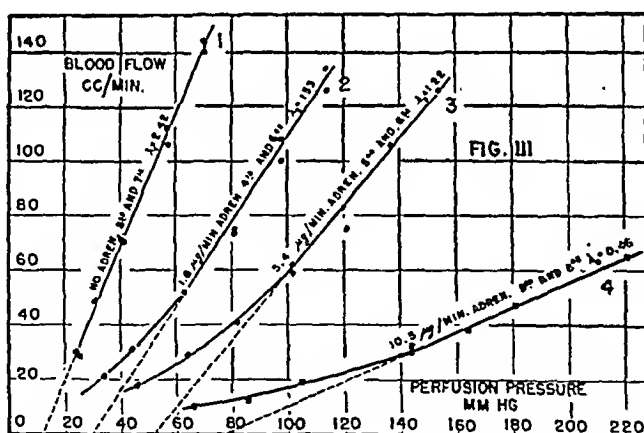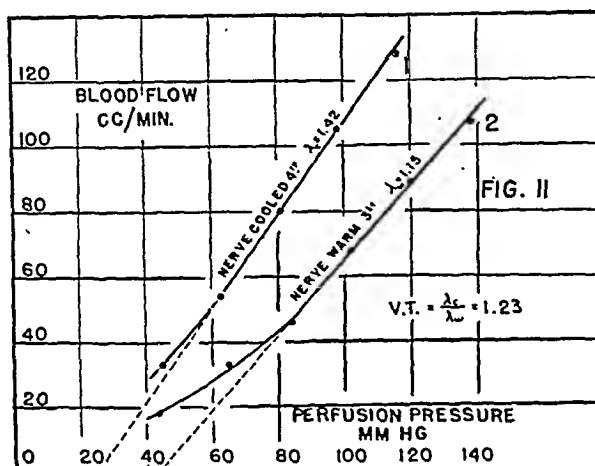
III. THE EFFECT OF VASOCONSTRICTION ON THE RELATION BETWEEN THE PRESSURE AND THE FLOW OF BLOOD AND OF RINGER'S SOLUTION. In the isolated perfused hindlimb in which the blood vessels were widely dilated by the addition of 0.1 per cent chloral hydrate to the perfusing blood, Whittaker and Winton (1933) found that the flow varied linearly with the pressure over a wide range of flows. They observed that if the linear region of the pressure-flow curve were extrapolated to zero flow it intercepted the pressure axis at about 20 mm. Hg. The value of this extrapolated intercept was shown to be increased when the viscosity of the blood was increased by the addition of red blood corpuscles.

A. EXPERIMENTAL. We have confirmed these findings in the isolated hindlimb muscles in which the blood vessels are unconstricted.[1] However, if vasoconstriction is produced the relations between the pressure and the flow of blood are altered. Figure 2 shows the effect on the pressure-flow curve caused by the vasomotor tone in the innervated preparation and figure 3 shows a family of such curves obtained from an isolated hindlimb in which vasoconstriction was produced by the continuous infusion of adrenalin into the perfusing blood. It is seen that the pressure-flow curves during vasoconstriction are linear only when the flows exceed certain values. Below these values the slopes diminish rapidly and the curves approach the origin. If the linear regions of these curves are extrapolated to zero flow (broken lines) they intercept the pressure axis at values which increase with increasing vasoconstriction (fig. 3). The effects of vasoconstriction on the pressure-flow curve of blood are therefore indistinguishable from the effects of an increase in corpuscular concentration. Dr. F. R. Winton informs us that he has observed a similar increase in the "intercept" when vasoconstriction is caused by pituitrin.

The effects of vasoconstriction caused by adrenalin on the flow of Ringer's solution are shown in figure 4. The data were obtained from the same experi-

---

[1] *Unconstricted* has been chosen in preference to *dilated* because the vessels of the isolated hindlimb may be actively dilated by various agents.

ment as that shown in figure 2 which illustrates the effects of vasoconstriction on blood. The double circulation technique was employed in order that the periods of Ringer perfusion might be limited to the few minutes required to obtain accurate pressure-flow data. That little edema occurred during the exposure to Ringer's solution is suggested by the fact that both lines intercept the pressure

Figs. 2, 3. The effects of vasoconstriction on the relation between the pressure and the flow of blood. Vasoconstriction caused by vasomotor tone in innervated preparation (fig. 2) and by adrenalin infusions in isolated preparation (fig. 3). *Note* 1, the curves are approximately linear at high flows and bend toward origin at low flows; 2, the effect of vasoconstriction is to increase pressure at which deviation from linearity occurs; 3, the extrapolated intercept on the pressure axis increases with vasoconstriction (broken lines); 4, reversibility as indicated by times at which points on each curve were determined.

axis near the origin. It is seen that no significant deviation occurred from a simple proportionality between pressure and flow. A similar experiment in which the vasoconstriction was caused by the vasomotor tone in the innervated preparation is shown in figure 6 (curves 1 and 2).

B. DISCUSSION. The implications of these findings which bear directly on the

problem of measuring quantitatively the degree of vasoconstriction may best be considered in terms of the resistance to flow.

The total resistance to flow is defined as the pressure per unit rate of flow. For the laminar flow of liquids through small tubes we have from Poiseuille's Law,

$$\text{Resistance} = \frac{P}{F} = \eta \times \frac{8l}{\pi r^4} \quad \dots\dots\dots\dots\dots\dots\dots\dots\text{(i)}$$

where P = pressure in dynes per cm.², F = flow in cm.³ per second, η = viscosity in dyne-seconds per cm.², l = length in cm. and r = radius in cm. It is seen that the resistance to flow is the product of two terms, (a) the internal (viscous) resistance of the liquid, (b) a factor which is determined by the dimensions of the tube. If the viscosity of the liquid is independent of the flow (homogeneous liquid) then a deviation from proportionality between pressure and flow indicates a change in the dimensions of the tube with flow as required by Equation i. If the dimensions of the tube are constant (rigid tube) then a deviation from proportionality between pressure and flow indicates a change in the viscosity of the liquid with flow. This type of change is characteristic of the flow of suspensions (Bingham 1922) and the viscosity at any particular flow is often termed the apparent viscosity.

1. *The resistance to the flow of Ringer's solution.* For any constant degree of vasoconstriction the flow of Ringer's solution has been found to be proportional to the pressure (fig. 4). This relation between pressure and flow is characteristic of the laminar flow of liquids through rigid tubes, and it implies that over the range of pressures investigated (10 to 160 mm. Hg) the average dimensions of the vascular elements responsible for the resistance to flow are not significantly altered. It implies further that the viscosity is independent of the flow and that at constant pressure a change of Ringer flow is a result solely of a change in the dimensions of the blood vessels. The ratio of the Ringer flow with the nerve blocked to the flow with the nerve active (constant pressure) may therefore be expected to be a quantitative measure of the effects of the vasomotor nerves on the average dimensions of the blood vessels.

2. *The resistance to the flow of blood.* In contrast to the flow of Ringer's solution the flow of blood is not proportional to the pressure. In the unconstricted vessels the deviation from proportionality is small and the pressure-flow curve reaches the linear part of its characteristic at pressures which are well below the physiological range (fig. 2, curve 1; fig. 3, curve 1; fig. 6, curve 3). During vasoconstriction the deviation from proportionality is greatly increased and the pressure-flow curve may not reach its final, approximately linear slope until pressures of 100 mm. Hg or more are exceeded (fig. 3, curves 3 and 4). Under these conditions the resistance to flow decreases greatly as the flow increases with pressure. For example, in figure 3, curve 3, the resistance at a flow of 20 cc./min. was 50/20 = 2.5 mm. Hg per cc. per min. whereas at a flow of 120 cc./min. the resistance was 150/120 = 1.25 mm. Hg per cc. per min. Since no corresponding changes occur with Ringer's solution (preceding paragraph), the decrease of resistance with flow of blood may be considered as a decrease in the internal resistance (apparent viscosity) of the blood itself. A change of blood flow brought about by vasoconstriction (at constant pressure) is therefore a result of a change in the apparent viscosity of the blood as well as of a change in the dimensions of the blood vessels

and the ratio at constant pressure of the blood flow in the unconstricted vessels to the flow in the constricted vessels (abbr. R_b) may be expected to vary with the pressure. Figure 5 shows the variation of R_b with pressure for each of the states of vasoconstriction shown in figures 2 and 3. Curve 4, figure 3, is not included because it cannot be conveniently plotted on the same scale. It is seen that as the pressure increases the rate of diminution of R_b with pressure decreases. At very high pressures ($p \rightarrow \infty$) the influence of the anomalous departures of the pressure-flow curves from linearity becomes negligible and R_b approaches the ratio of the slope of the pressure-flow curve in the unconstricted vessels to the
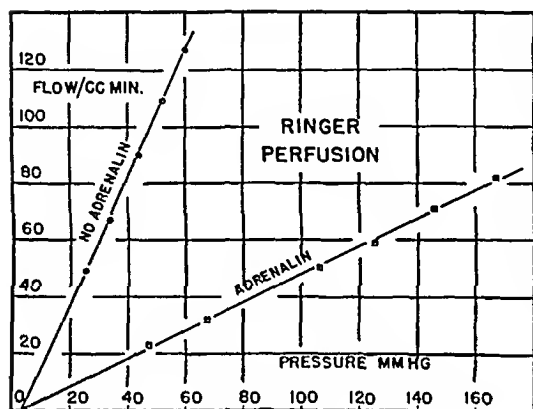

Fig. 4.

Fig. 5.

Fig. 4. The effects of vasoconstriction on the relation between pressure and flow of Ringer's solution. *Note* 1, that both curves are straight lines going through a point near the origin; 2, that the slope is in each case the reciprocal of a resistance which is independent of the flow at which it is measured.

Fig. 5. The variation with pressure of the ratio of the blood flow in the unconstricted vessels to the flow in the constricted vessels. The curves depict three different constant degrees of vasoconstriction plotted from the data of figures 2 and 3. In the physiological range of pressures R_b varies with pressure and cannot therefore be used as a measure of the vasomotor tone. With increasing pressure R_b approaches a limit which is equal to the ratio of the slopes of the pressure-flow curves (1.23, 1.58 and 1.98 in the examples shown above). This limit is proposed as a quantitative measure of the degree of vasoconstriction as explained in text.
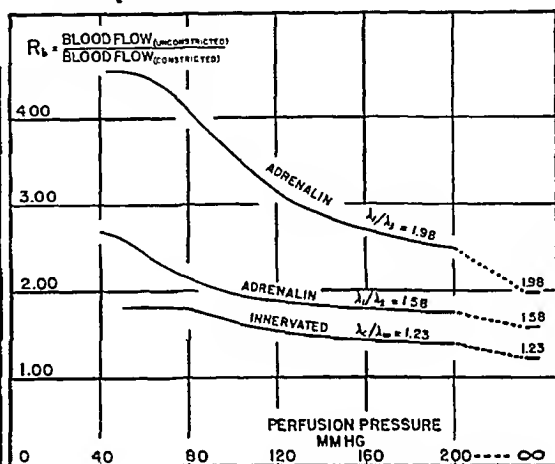
slope of the pressure-flow curve in the constricted vessels. Under these conditions we thought it probable that R_b would be determined solely by the change in the dimensions of the blood vessels. If this is the case then for any constant degree of vasoconstriction the ratio of the slopes of the pressure-flow curves on Ringer in the unconstricted and in the constricted blood vessels should be equal to the ratio of the slopes of the pressure-flow curves on blood in the unconstricted and in the constricted vessels, both slopes being measured at high pressures over the linear parts of the pressure-flow curves.

IV. Evidence that during any constant degree of vasoconstriction the ratio of the Ringer flows at constant pressure is approximately

EQUAL TO THE RATIO OF THE SLOPES OF THE PRESSURE-FLOW CURVES ON BLOOD MEASURED OVER THE LINEAR PARTS OF THEIR CHARACTERISTICS. In four out of five experiments performed to test this hypothesis·the double pump-lung innervated preparation was used and different states of vasomotor tone were induced by bleeding the animals. The last experiment was performed on the isolated curarized limb and vasoconstriction was produced by stimulating the sciatic nerve with 60 cycle A.C. from a constant voltage source. The design of each experiment was as follows:

1. Pressure-flow curves of six or more points each were obtained on blood with the nerve warm until a steady state was reached.

2. The nerve was cooled and a pressure-flow curve obtained.

3. The nerve was warmed and one or two points taken to ascertain whether the vasomotor tone was steady.

4. The limb was "switched over" to the Ringer circulation and pressure-flow curves of two or three points each were obtained as quickly as possible in the following order: a, innervated; b, nerve cooled; c, innervated.

5. The blood circulation was restored and steps (1) and (2) repeated.

In the electrical stimulation experiment this sequence was modified to avoid exposing the limb to Ringer's solution during the period of recovery from stimulation. For this reason comparisons of the Ringer flows in the constricted and unconstricted vessels were made at rather long intervals and are not as reliable as in other experiments.

Owing to the number of data required we were unable to reduce the periods of Ringer perfusion to less than $\frac{1}{2}$ hour and edema developed in some experiments. The development of edema manifests itself in the pressure-flow curve by a gradual reduction of slope which in the case of Ringer perfusion affects both the constricted slope and the unconstricted slope in the same proportion. Since we are primarily concerned with ratios such changes are not of critical importance. More serious are real changes of vasomotor tone which may have occurred during the measurements. Such changes would critically affect the results if they occurred between steps (3), (4a) and (4b) and these procedures generally required 15 minutes to complete. In the absence of direct evidence as to whether or not such changes occurred we can only refer to the consistency of the results and the fact that in three of the experiments the vasomotor tone measured by the ratio of the slopes of the pressure-flow curves on blood was the same before and after the cycle of measurements.

The best of these experiments is illustrated in figure 6. It is seen that the pressure-flow curve on blood with the nerve.warm was the same before, during, and after the steps outlined above. Data from all the experiments are shown in table 1. Without exception the ratio of Ringer flow to blood flow at constant pressure (apparent viscosity) was increased as a result of vasomotor activity. With the exception of the last experiment which for reasons given above was technically the least satisfactory, the ratio of the Ringer flows approximated the ratio of the slopes of the pressure-flow curves on blood. The average of our 7 measurements of the apparent viscosity at a pressure of 80 mm. Hg with the

vessels unconstricted was 2.5 as compared with the average value of 2.2 found under similar conditions by Whittaker and Winton (1933). During vaso-constriction caused by vasomotor nerves, however, the apparent viscosity measured in the same way averaged 3.6. According to the data of Whittaker and Winton this would correspond with a corpuscular concentration of 70 per cent in the isolated preparation.

The results of the double circulation experiments shown in table 1 enable us with some confidence to make the following computations from the characteristics of the pressure-flow curves on blood.

1. The ratio of the total resistance to blood flow at constant pressure with and without vasoconstriction $= \dfrac{P_o}{\text{Flow (constricted)}} \div \dfrac{P_o}{\text{Flow (unconstricted)}} = R_b.$
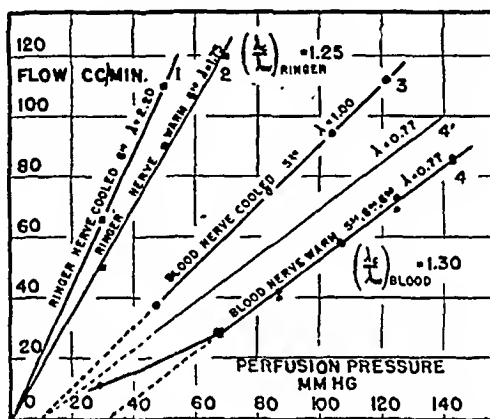
Fig. 6. Evidence that during any constant degree of vasomotor tone the ratio of the Ringer flows at constant pressure (1.25) is approximately equal to the ratio of the slopes of the pressure flow curves of blood measured over the linear parts of their characteristics (1.30). Double pump-lung innervated preparation. *Note* 1, reversibility of experiment as indicated by times at which points on curves were obtained; 2, the pressure-flow curve of blood (4′) which would have resulted had there been no change of apparent viscosity.

2. The ratio of the Ringer flows at constant pressure without and with vasoconstriction. This quantity is independent of the pressure (fig. 4) and of the viscosity (p. 192). For reasons given in the discussion we propose to adopt it as a measure of the vasomotor tone (V.T.).

$V.T. = R_r \equiv \lambda c / \lambda w$ where c and w refer to nerve cooled and nerve warmed respectively

3. The ratio of the apparent viscosities of the blood at constant pressure with and without vasoconstriction.

$$\frac{\eta_w}{\eta_c} = \frac{R_b}{R_r} \equiv \frac{R_b}{V.T.}$$

V. STATISTICAL. The effects of vasoconstriction on the pressure-flow curves of blood have been studied in 16 hindlimbs. Nineteen pairs of curves were obtained from innervated preparations in which the vasomotor tone ranged

from 1.2 to 2.5. Eight sets of curves were obtained from isolated preparations in which the tone ranged from 1.1 to 5.4.

Without exception vasoconstriction increased the apparent viscosity of the blood. However, the percentage increase of apparent viscosity associated

TABLE 1

Comparison of Ringer pressure-flow curves with blood pressure-flow curves

Double pump-lung innervated perfused hindlimb preparation

R_b = Blood flow (unconstricted) ÷ Blood flow (constricted).
R_r = Ringer flow (unconstricted) ÷ Ringer flow (constricted).
λ = Slope of pressure-flow curve over linear part of characteristic.
η = Apparent viscosity of blood (Ringer flow ÷ Blood flow).
$0, 1$ = Subscripts referring to unconstricted and constricted vessels respectively.

EXPERIMENT NUMBER AND TYPE	PERF. PRESS.	R_b	R_r	λ_0/λ_1	η_0	η_1
	mm. Hg					
1. Isolated	40				3.1	
	80				2.1	
	120				1.9	
2. Innervated	40	2.7			2.0	3.0
	80	2.0	1.8	1.6	1.8	2.0
	120	1.8			1.7	1.8
3. Innervated	80	3.0	2.6	2.2	2.7	3.1
	120	2.6			2.5	2.5
4. Innervated	40	1.6			3.5	4.2
	80	1.5	1.4	1.3	2.9	3.1
	120	1.4			2.7	2.8
4. Innervated	40	2.1			2.8	4.7
	80	2.0	1.25	1.3	2.5	3.9
	120	1.7			2.3	3.1
5. Innervated	40	3.2			3.4	7.6
	80	2.3	1.4	1.3	3.0	4.9
	120	1.8			2.8	3.1
6. Electrical stim.	40	2.4			2.7	5.0
	80	2.5	1.35	2.0	2.5	4.4
	120	2.2			2.4	4.0

Mean and standard deviation at pressure of 80 mm. Hg (average hematocrit = 40%):
$\eta_0 = 2.5 \pm .4.$　$\eta_1 = 3.6 \pm 1.0.$

with any particular degree of vasoconstriction varied widely from one hindlimb to another. Thus in two preparations with a vasomotor tone of 1.30 the percentage increase of apparent viscosity at a pressure of 80 mm. Hg was in one case 21 per cent, in the other 80 per cent. For the purpose of assembling the data in compact form we have constructed table 2 without reference to

differences in vasomotor tone. The standard deviations thus include differences resulting from different vasomotor tones as well as deviations observed from one preparation to another. The means are therefore useful only for orders of magnitude and for relative changes of viscosity with pressure.

VI. DISCUSSION. 1. *The changes of apparent viscosity.* The dimensions of the vascular bed in the hindlimb muscles perfused under the conditions of our experiments are evidently such that even a small degree of vasoconstriction is sufficient to increase the apparent viscosity of the blood. The sympathetic nerves appear to owe much of their effectiveness in the control of the blood flow to the anomalous physical properties of the blood. The effect on the blood flow of a given change in the average dimensions of the blood vessels is augmented by the simultaneous change in the apparent viscosity and the amount of change is greatest at low pressures where an increase of peripheral resistance becomes of greatest importance to the animal. During the vasoconstriction accompanying hemorrhage, for example, the effective increase of

TABLE 2

The effects of vasoconstriction on the apparent viscosity of the blood

Data compiled from 24 sets of pressure-flow curves obtained from 16 hindlimbs in which the vasomotor tone was 1.0–2.5.

Means, standard deviations, and deviations of means.

PERFUSION PRESSURE	PER CENT INCREASE OF APPARENT VISCOSITY	PER CENT OF TOTAL CHANGE OF RESISTANCE DUE TO INCREASE OF APPARENT VISCOSITY
mm./Hg		
40	78 ±62 (±18)	39 ±18 (±5)
60	61 ±47 (±11)	40 ±18 (±5)
80	49 ±36 (± 4)	35 ±18 (±4)
100	34 ±18 (± 4)	30 ±16 (±3)
120	25 ±17 (± 4)	26 ±15 (±3)

resistance of the perfused muscles is often double that which would occur without change of apparent viscosity.

It was noted by Whittaker and Winton (1933) that the absolute value of the blood flow through isolated hindlimbs at any given pressure varies greatly from one preparation to another although the apparent viscosity of the blood is relatively independent of such variations. The results of the present experiments imply that these differences of flow are due to differences in the number of perfused vascular elements rather than to differences in their average dimensions, for in the latter case considerable variations in the apparent viscosity would be expected. For example, in two isolated hindlimbs, A and B, the blood flows measured at a pressure of 130 mm. Hg were 150 cc./min. and 52 cc./min. per 10 kgm. dog respectively. The apparent viscosity of the blood was in each case approximately the same as indicated by the pressure-flow curves. Vasoconstriction was then produced in A until the blood flow measured at 130 mm. Hg was the same as that in B (52 cc./min.). As a result of the vasoconstriction the apparent viscosity of the blood was increased 70 per cent.

The finding that the apparent viscosity of the blood is increased during vasoconstriction appears to conflict with the observations of Fahraeus and Lindquist (1931) on the flow of blood in glass capillaries. These authors found that at a pressure of 100 mm. Hg the apparent viscosity of the blood was decreased as the diameter of the capillary was decreased from 0.3 to 0.04 mm. However, they found it "impossible to press the blood through still narrower tubes," and presumably the apparent viscosity was increased in this range. It seems desirable that the factors affecting the flow of blood through small capillaries be reinvestigated over a wide range of pressures.

2. *The quantitative measure of the vasomotor tone.* Evidence has been obtained that the ratio of the slopes of the pressure-flow curves of blood in the unconstricted and constricted vessels of the hindlimb muscles may be considered as equivalent to the ratio of the slopes of the pressure-flow curves of Ringer's solution under the same conditions. The evidence suggests that this ratio is independent of the viscosity and depends solely upon the change in the average dimensions of the blood vessels (p. 193). In the innervated preparation the ratio of the slope with the nerve blocked and with the nerve active has been obtained and in this case the ratio may be considered as a measure of the vasomotor tone. Its value has been found to vary from 1.0 (no vasomotor tone) to about 3.5 (large tone produced by hemorrhage) and it may be measured within an error of about ±10 per cent.

Reasons have been given above for supposing that the average dimensions of the unconstricted vessels are not greatly different in the muscles of different dogs. A change in the average dimensions of the blood vessels brought about by any particular degree of vasoconstriction would therefore be expected to be the same in the muscles of different animals. This implies that the effects on the vasomotor tone (measured as described above) of any particular experimental procedure are quantitatively comparable in the muscles of different experiments.

SUMMARY

1. The hindlimb muscles of anesthetized dogs were perfused with defibrinated blood at constant pressure from a pump-lung circulation. The sciatic nerve to the muscles was left intact. At intervals the nerve was reversibly blocked by cooling and the changes of blood flow measured at different perfusion pressures. The blood perfusion could be interrupted by periods of Ringer perfusion.

2. With the blood vessels unconstricted (nerve blocked) the relations between the pressure and the flow of blood or of Ringer's solution were similar to those found by Whittaker and Winton (1933) in the isolated hindlimb (figs. 2, 3, 4, 6).

3. During vasoconstriction the following changes occur in the pressure-flow curves: a. The pressure at which the pressure-flow curve of blood becomes approximately linear is increased. Below this pressure the slope diminishes and the curve approaches the origin. The extrapolated intercept of the linear

part of the curve increases with increasing vasoconstriction (figs. 2, 3, 6). *b.* The Ringer pressure-flow curve is a straight line which intercepts the pressure axis at or near the origin. Its slope is diminished by vasoconstriction but its intercept is unaffected (figs. 4, 6).

4. The apparent relative viscosity of the blood (ratio of Ringer flow to blood flow at constant pressure) is increased during vasoconstriction. The amount of increase varies in different muscles and in the same muscles with the degree of vasoconstriction and with the pressure (fig. 5). Extreme values for blood of normal corpuscular concentration are 2 to 8. At normal pressures the change of apparent viscosity accounts for about $\frac{1}{3}$ of the total change of resistance to blood flow caused by vasoconstriction (table 2).

5. For any constant degree of vasoconstriction the ratio of the Ringer flows at constant pressure in the unconstricted and in the constricted blood vessels is approximately equal to the ratio of the slopes of the pressure-flow curves of blood in the unconstricted and in the constricted vessels, both slopes being measured over the linear parts of their characteristics.

6. The evidence suggests that this ratio is independent of the viscosity and is a measure of the change in the average dimensions of the blood vessels. In the innervated preparation it is proposed as a measure of the vasomotor tone. Its value has varied from 1.0 (no vasomotor tone) to about 3.5. Reasons are given for supposing that the measure is quantitatively comparable in the muscles of different experiments.

REFERENCES

BINGHAM, E. C. Fluidity and plasticity. Chapt. VII. McGraw-Hill, New York, 1922.
EGGLETON, M. G., J. R. PAPPENHEIMER AND F. R. WINTON. J. Physiol. 97: 363, 1940.
FAHRAEUS, R. AND T. LINDQUIST. This Journal 96: 562, 1931.
GADDUM, J. H. J. Physiol. 67: 16P., 1929.
KRAMER, K. AND F. R. WINTON. J. Physiol. 96: 87, 1939.
PAPPENHEIMER, J. R. J. Physiol. 99: 283, 1941.
POISEUILLE, J. L. M. Extraits du Tome IX des Savants Etrangers, Académie des Sciences, Paris, 1844.
WHITTAKER, S. R. F. AND F. R. WINTON. J. Physiol. 78: 339, 1933.

EFFECTS OF PURIFIED PITUITARY PREPARATIONS ON THE NONPROTEIN NITROGEN CONSTITUENTS OF BLOOD[1]

JANE FRAENKEL-CONRAT,[2] HEINZ FRAENKEL-CONRAT
AND HERBERT M. EVANS

From the Institute of Experimental Biology, University of California, Berkeley

The important rôle of the pituitary in nitrogen metabolism has been evident since the recognition that growth in higher animals is largely controlled by this gland. The pioneer work of Teel and Watkins (1, 2), Gaebler (3–5), and Lee and his associates (6–9), has clearly demonstrated that the gain in body weight which can be produced with pituitary implants or extracts is associated with a decrease in nitrogen excretion and with a lowering of the concentration of blood and tissue nonprotein nitrogen constituents. All these changes were generally regarded as caused by the growth hormone, although rigidly purified growth-promoting preparations had never been used for such studies. The development in this laboratory of methods for the preparation of the six "accepted" pituitary hormones in comparatively pure state has enabled us to investigate the action of each of these on the various constituents of the nonprotein nitrogen of rat blood. A similar study of the action of the purified hormones on urinary nitrogen excretion has been performed by W. Marx (10).

EXPERIMENTAL MATERIAL, CONDITIONS AND METHODS. *Animals.* Normal plateaued female rats were used for the major portion of these experiments, approximately 650 in all, many of which had previously been used for assay of growth hormone. However, if at least a week were allowed to elapse between their last injection for growth hormone assay and use in these experiments, such rats were found not to differ from previously unused rats in the nitrogenous components of their blood and in their responsiveness to hormone treatment. All rats were fasted 28 hours before blood samples were taken for analysis. Injections were made intraperitoneally, generally in 1 cc. doses, 4 hours before blood samples were taken.

About 120 hypophysectomized animals of several types were also employed. Normal plateaued female rats were hypophysectomized 2 days before being sacrificed for blood samples. Another group of plateaued female rats was hypophysectomized 2 weeks prior to the experiment, and still another group was about 6 weeks postoperative when used. The male hypophysectomized rats were 2 months old at operation and 1 month postoperative at the time of the experiment. The completeness of operation was checked in all cases at autopsy and only completely hypophysectomized animals were included in the tables. To counteract

[2] In partial fulfillment of the requirements for Ph.D. degree.

the hypoglycemic tendency of fasting hypophysectomized animals, glucose was administered once or twice during the fasting period.

Three groups of female rats were thyroidectomized between 2 and 2.5 months of age and the experiments performed when they were 4 months old. The completeness of the operation was checked by determination of their basal metabolic rate, part being done in a Benedict apparatus, and part in a modified Haldane set-up. The retardation of growth of the operated rats was used as an added index to the completeness of operation. In all doubtful cases the thyroid region was examined under the binocular at autopsy and sections made for histological examination. Those animals considered to be incompletely thyroidectomized were not included in compilation of results. Sixty-two completely thyroidectomized animals were used.

The hormone preparations used in this investigation were similar to those employed in other metabolic studies carried out in this laboratory (11). The lactogenic hormone and part of the interstitial-cell stimulating hormone (ICSH, LH) fractions used were physico-chemically pure. The adrenocorticotropic hormone contained some lactogenic activity. Part of the growth hormone preparations gave evidence of adrenocorticotropic hormone contamination, but all contained less than 1 per cent of other known hormones. This purification was achieved by cysteine treatment and isoelectric precipitations (12, 13). The growth potency ranged from 20 to 50 units per mgm. The thyrotropic preparations were contaminated only with ICSH (10–20 per cent). The dose necessary to produce body-weight gains in hypophysectomized rats indicated about 1 per cent of growth hormone contamination in these thyrotropic preparations. However, in view of the fact that similar weight gains can be produced by thyroxin in such rats (11) and also in view of the well established synergism between growth hormone and thyroxin (14) or thyrotropic hormone (15), this apparent growth hormone contamination of 1 per cent can only be regarded as a maximum figure. The thyrotropic fractions were prepared in the usual manner (16) and contained about 50 chick units per mgm.

Methods. Blood was drawn from the inferior vena cava of animals under ether anesthesia, sodium oxalate being used as anticoagulant. Three cubic centimeters of blood were taken to allow for separate determination of urea, amino acids, and nonprotein nitrogen and analysis of other nitrogenous substances upon the remaining filtrates which were pooled according to groups. Separate samples of whole blood were used for determination of hemoglobin and serum protein.

Preparation of protein-free filtrates was carried out by the method of Folin and Wu (17). More filtrate was obtained if centrifuging were substituted for the filtration recommended. The determination of alpha-amino acids was made according to the method of Folin (18), color intensity however being determined by means of a photoelectric colorimeter (Klett). Nonprotein nitrogen was determined by the microKjeldahl method. Urea was determined by the distillation method of Folin and Svedberg (19) with a slight modification, namely, that the receiver contained 4 cc. 0.01 N HCl, the remaining acid being back-titrated

with 0.01 N NaOH. On the combined filtrates total creatinine was determined by the method of Rose, Helmer, and Chanutin (20), and uric acid according to the method of Folin (21).

Peptide nitrogen was determined by 2 different methods. That given by Snell (22) permitted estimation of higher peptides by the difference in the nitrogen content of phosphotungstic and trichloracetic acid filtrates of the serum. Nitrogen content of these filtrates was determined by microKjeldahl. The lower peptides present in the usual tungstic acid filtrate were determined by taking the difference in alpha-amino acids before and after hydrolysis. Hydrolysis was carried out by heating 4 cc. aliquots of filtrate with 0.5 cc. concentrated sulfuric acid for 8 hours at 133°C. in an oil bath. Solutions were neutralized with 70 per cent sodium hydroxide and the Folin procedure was followed for amino acid determination.

Whole blood was used for the determination of hemoglobin concentration by the method of Sanford, Sheard and Osterberg (23). Total serum protein was determined by microKjeldahl method.

Muscle (gastrocnemius) and liver protein-free filtrates were prepared according to the method of Schafer and Lee (9). The determinations of the various nitrogen constituents were carried out as on the blood filtrates.

The authors are indebted to Dr. W. O. Reinhardt for performing most of the thyroidectomies and to Miss F. Carter for the hypophysectomies; also to Mr. V. V. Herring for assistance in the collecting of blood samples and for the determinations of oxygen consumption by the Haldane apparatus. The purified ICSH, lactogenic and part of the growth hormone preparations were kindly supplied by Drs. C. H. Li, W. R. Lyons and W. Marx, respectively.

RESULTS AND DISCUSSION. The reader is referred to the tables for detailed results. Tables 1 to 3 give results of representative experiments and tables 4 to 6 summarize all the data obtained during these studies. It is evident that crude alkaline extracts of beef anterior pituitary and globulin fractions prepared from such extracts caused the expected decreases in the blood nonprotein nitrogen, and in urea and amino acids in particular (table 1, 1 a, b; table 4, 1, 2). Purified growth hormone, however was found not to cause a change in blood urea under the conditions of these experiments[3]; it produced smaller decreases in amino acids than elicited by cruder extracts; insignificant decreases in nonprotein nitrogen, corresponding and probably due to those of the amino acids were observed (table 1, 2 a; table 4, 3).

In search for the active principle contained in the unfractionated extracts, the other known pituitary hormones were tested, i.e., thyrotropic, lactogenic, adrenocorticotropic, and the gonadotropic hormones. Of these, only thyrotropic hormone was found to be effective. This hormone was regularly observed to cause, within four hours, decreases in blood nonprotein nitrogen and in particular in blood urea; it also caused a drop in amino acids, at smaller doses than are

[3] Recent results indicate that growth hormone causes decreases in blood urea, more strikingly in hypophysectomized than in normal rats, when the hormone is administered for longer time periods.

needed in the case of the globulin fraction or purified growth hormone preparations (table 1, 2 b, c, 4 a, 5 a; table 4, 4).   No significant decreases in the non-

TABLE 1

Effects of various hormones on blood nonprotein nitrogen constituents of normal rats

EX-PERI-MENT NUM-BER	HORMONE PREPARATION		NPN			UREA-N			AMINO ACID-N		
	Type of hormone	Dose	Average	Change	S*	Average	Change	S	Average	Change	S
Experiments 1–4: Injected 4 hours before taking of blood samples (10 rats per group)											
		mgm.	mgm. per cent	per cent		mgm. per cent	per cent		mgm. per cent	per cent	
a	Globulin fraction	4.0	32.2	−13	s	14.3	−25	hs	6.9	−23	hs
1 b	Globulin fraction	0.5	34.6	−7	ns	15.2	−20	hs	8.3	−8	s
c	Adrenocorticotropic	2.0	41.6	+12	ns	21.5	+13	ns	9.0	0	
d	Controls		37.2			19.1			9.0		
a	Growth	3.0	41.3	−3	ns	13.6	0		7.0	−20	hs
2 b	Thyrotropic	3.0	36.8	−14	hs	8.7	−33	hs	7.0	−20	hs
c	Thyrotropic	0.5	36.8	−14	hs	11.2	−19	hs	7.0	−20	hs
d	Controls		42.6			13.6			8.7		
3 a	Lactogenic	2.0	36.6	−1	ns	11.7	−3	ns	8.9	+11	hs
b	Controls		36.9			12.0			8.0		
a	Thyrotropic	1.0	37.6	−9	s	10.4	−29	hs	7.6	−9	hs
4 b	Thyroxin	0.1	38.8	−6	ns	12.9	−12	ns	7.6	−9	hs
c	Thyroxin	0.02	40.0	−3	ns	13.9	−5	ns	7.9	−5	s
d	Controls		41.4			14.7			8.3		
Experiment 5: Injected 5 times within 52 hours preceding taking of blood samples† (12 rats per group)											
a	Thyrotropic	2.5	33.2	−8	ns	11.7	−27	hs	7.9	−4	ns
5 b	Thyroxin	0.25	35.7	−1	ns	12.8	−21	s	8.7	+6	ns
c	Controls		36.1			16.1			8.2		

* S denotes Statistical Significance of the difference between the mean of treated and control groups; s = significant, hs = highly significant and ns = not significant; Standard Errors of the Differences between Means was calculated according to formulas for small samples (Outline of Statistical Methods, H. Arkin and R. R. Colton, 4th Ed. College Outline Series, p. 127).   Following the usual table for t values, differences which approached or exceeded 3 times their error were called highly significant, those in which this ratio was between about 2.1 and 2.9 significant, the rest not significant.

† Administered one fifth the dose at 9 a.m. and 3 p.m. on first and second day and at 9 a.m., 4 hrs. preceding autopsy on the third day.   Limited and identical amounts of food given on first day, fasted on second day; Urine N determined by W. Marx (10): First day: Thyrotropic Hormone 176 mgm., Thyroxin 218 mgm., Controls 228 mgm.; on second day: 137, 176, 186 mgm. respectively.

protein nitrogen constituents were obtained with the other purified pituitary hormones (table 1, 1 c, 3 a; table 4, 5–7).

With the surprising finding that thyrotropic hormone appeared to be primarily responsible for the early blood nonprotein nitrogen changes[3] provoked by pituitary extracts, the question presented itself whether this action was a, mediated by the thyroid gland, or b, due to a direct action of the thyrotropic hormone, or c, caused by another hormone regularly contaminating the former. To determine whether the action of the thyrotropic hormone was mediated by the thyroid, two types of experiments were performed: the action of thyroxin was studied, as well as the effect of thyrotropic hormone in thyroidectomized rats. As indicated in the tables (table 1, 4 b, c; table 4, 8), thyroxin caused a drop in blood urea, though to a less marked degree than thyrotropic hormone, in the four-hour test. Since thyroxin is known to act very slowly, a number of experiments were performed in which this hormone was allowed to act for 24 and 52 hours. It was then found to lower blood-urea levels almost as effectively as did the thyrotropic hormone, while its effect on blood amino acids was doubtful[4] (table 1, 5 b).

Conversely, the administration of thyrotropic hormone to thyroidectomized rats caused only insignificant decreases in blood urea, while still decreasing blood amino acids[5] (table 2; table 5, 1). Both the action of thyroxin in normal rats and that of thyrotropic hormone in thyroidectomized rats seem to indicate that the latter affects blood urea through stimulation of the thyroid while its action on amino acids may be direct.

From these experiments it appears that the action of thyrotropic hormone on blood nonprotein nitrogen constituents is mainly, but not entirely, mediated by the thyroid gland. The question still remains whether that part of its effect (primarily on the amino acids) which can be elicited also in the absence of the thyroid is due to a direct action of the thyrotropic hormone per se or to another factor contaminating thyrotropic preparations. The only known contaminants

[4] Thyroxin was administered only for short periods of time (4–52 hrs.) and at comparatively low doses (0.02–0.25 mgm.). This was done since it was intended to duplicate the conditions under which low doses of thyrotropic hormone elicit certain effects within four hours. It is recognized that the physiological effects of thyroxin observed under such conditions may be very different from those characterizing the hyperthyroid state. This was indicated when in one experiment blood nonprotein nitrogen values of hyperthyroid rats were compared with those of controls (0.04–0.05 mgm. thyroxin daily for 11 days; O_2 consumption 25 per cent above controls); nonprotein nitrogen was found increased (+12 per cent), changes in urea and amino acid nitrogen not significant. Similar differences between the physiological action of thyroxin and hyperthyroidism have been demonstrated to occur in regard to growth (14), pancreatic insulin content (24), etc.

[5] The oxygen consumption of apparently completely thyroidectomized rats ranged from 98 to 131 l./m.²/24 hrs. No correlation between the oxygen consumption and the blood nitrogen values could be observed. In one experiment a new method for determining the completeness of thyroidectomy was applied by W. O. Reinhardt (25). This consists in treating the operated rats with radioactive iodine, then preparing radio-autographs of the entire region in which remnants or accessory thyroid tissue might be expected. By this procedure small centers of iodine concentrating tissue were found in a number of rats which appeared to be completely thyroidectomized by all other criteria. The effect of the hormone on rats which were free from thyroid tissue also according to this most sensitive test indicated essentially the same as in animals with traces of thyroid, i.e., a lowering of the amino acids, but no consistent effect on nonprotein nitrogen or blood urea.

of such preparations are the growth hormone and one of the gonadotropins, the interstitial-cell-stimulating hormone (ICSH or LH). As previously stated, most of the thyrotropic samples used in these studies may have contained approximately one per cent of the former and 10 to 20 per cent of the latter. However, it does not appear likely that any part of the action of thyrotropic hormone on the blood nonprotein nitrogen could be due to either of these contaminants since

TABLE 2

Effect of thyrotropic hormone on blood nitrogen constituents of thyroidectomized and normal rats*

DETERMINATION	THYROIDECTOMIZED RATS				NORMAL RATS			
	Injected		Control		Injected		Control	
	Average	Change	Average	Change	Average	Change	Average	
		per cent		per cent		per cent		
Non-protein-N...	38.8 mgm.%	−9 (s)	42.7 mgm.%	+6 (s)	35.0 mgm.%	−13 (hs)	40.2 mgm.%	
Urea-N..........	18.4 mgm.%	−11 (ns)	20.7 mgm.%	+30 (hs)	13.1 mgm.%	−9 (ns)	14.4 mgm.%	
α-Amino-acid-N†	7.5 mgm.%	−12 (s)	8.5 mgm.%	−25 (hs)	9.2 mgm.%	−19 (hs)	11.4 mgm.%	
Peptide-N Serum poly-peptide-N....	7.8 mgm.%	−58	18.6 mgm.%	−10	15.9 mgm.%	−23	20.7 mgm.%	
Filtrate peptide-N.......	1.5 mgm.%	−73	5.6 mgm.%	+27	4.2 mgm.%	−5	4.4 mgm.%	
Total creatinine-N..........	2.6 mgm.%	−11	3.0 mgm.%	+36	2.3 mgm.%	+5	2.2 mgm.%	
Uric acid-N......	0.2 mgm.%	0	0.2 mgm.%	−33	0.2 mgm.%	−33	0.3 mgm.%	
Serum protein-N	1.09%	0	1.09%	0	1.05%	−5	1.09%	
Hemoglobin......	16%	−27	22%	−4	31%	+35	23%	

* Each group contained 10 female rats (8 in thyroidectomized control group), 114 days old. Thyroidectomies were performed on day 72–74. Oxygen consumption of the completely thyroidectomized rats averaged 120 l./m.²/24 hours, which is 28 per cent below that of normal rats. The injected rats received 1 mgm. thyrotropic hormone 4 hours before the taking of blood samples. The letters in parentheses denote statistical significance.

† The amino acids in this experiment were determined without use of the Klett photoelectric colorimeter and are therefore not directly comparable with the absolute values of all other experiments which were always lower.

pure ICSH is without effect (table 4, 7) and growth hormone does not affect blood urea at all under conditions favorable for the action of the thyrotropic hormone, while lowering the amino acids only at somewhat higher levels than are needed of the thyrotropic hormone (table 1, 2 a; table 4, 3). It is recognized that part of the blood action might be due to an unknown hormone present in thyrotropic preparations; but in the absence of any evidence favoring this, it appears preferable to assume that all the observed effects are really due to the

thyrotropic hormone, an assumption which can ultimately be proven or disproven only by the isolation of the pure hormone.

As in all studies of the action of pituitary hormones it appeared also here important to use hypophysectomized animals (table 5, 2). Thus when such rats were used only two days after the operation, they were found to react similarly to normal rats although great variations occurred within the group. After a longer postoperative period, however, the blood nonprotein nitrogen, and especially the

TABLE 3

Effect of thyrotropic hormone and thyroxin on blood, liver and muscle nonprotein nitrogen constituents of hypophysectomized and normal rats*

DETERMINATIONS	HYPOPHYSECTOMIZED RATS						NORMAL RATS				
	Thyrotropic (6 rats)		Thyroxin (7 rats)		Control (7 rats)		Thyrotropic (10 rats)		Thyroxin (10 rats)		Controls (10 rats)
	Average	Change	Average	Change	Average	Change	Average	Change	Average	Change	Average
	mgm. per cent	*per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*
Blood:											
N-P-N	45.7	−47 (hs)	36.2	−54 (hs)	78.6	+81 (hs)	38.1	−12 (hs)	37.4	−14 (hs)	43.3
Urea-N	25.3	−52 (hs)	14.3	−73 (hs)	52.7	+230 (hs)	13.9	−13 (hs)	12.7	−21 (hs)	16.0
α-Amino acid N	6.3	−7 (ns)	7.3	+7 (ns)	6.8	−20 (hs)	7.5	−12 (hs)	8.2	−4 (ns)	8.5
Liver:†											
N-P-N	197.0	−25	239.0	−7	258.0	+25	202.0	−2	156.0	−25	207.0
Urea-N	34.2	−47	34.1	−47	64.9	+116	29.0	−3	22.9	−24	30.0
α-Amino acid-N	59.8	−14	78.2	+12	69.6	+5	62.0	−3	55.0	−14	63.6
Muscle:†											
N-P-N	307.0	−12	303.0	−13	348.0	+12	307.0	−1	279.0	−10	310.0
Urea-N	33.6	−46	25.3	−59	61.8	+147	24.6	−1	22.0	−12	25.0
α-Amino acid-N	74.7	−1	70.6	−6	75.0	+9	67.8	−2	65.0	−6	69.1

* Thyrotropic hormone (1 mgm.) was given 4 hours before autopsy, thyroxin (0.25 mgm.) 24 hours before autopsy. The rats were plateaued females (ca. 6 months old), hypophysectomies having been performed 2 weeks prior to the experiment.

† Livers and muscles were pooled in groups and analyses performed in duplicate or triplicate.

blood urea, of untreated rats was found to be very much higher and the effect of thyrotropic hormone and thyroxin in decreasing these levels much more pronounced in hypophysectomized than in normal rats[6] (table 3; table 5, 2; table 6).

[6] Histological examination of the thyroids of hypophysectomized rats, which had received thyrotropic hormone four hours before autopsy, did not reveal any signs of stimulation. This is not surprising in view of the short period of treatment; on the other hand, this finding does not elucidate the mechanism of the striking effects on blood non-protein nitrogen produced by thyrotropic hormone under these conditions.

TABLE 4

Summary of the effects of hormones on blood nonprotein nitrogen constituents of normal rats
(4-hour injection period)

HORMONE PREPARATION		CHANGE, COMPARED WITH RESPECTIVE CONTROLS*		
Type of hormone	Dose	NPN	Urea N	Amino acid N
	mgm.	*per cent*	*per cent*	*per cent*
1. Crude alkal. extr.	5.0			−10 (6)
	2.5	−10 (6)		−13 (18)
	1.0			+7 (6)
2. Globulin fraction	4.0	−13 (10)	−25 (10)	−23 (10)
	3.0			−18 (4)
	2.5			−13 (8)
	2.0	−19 (10)		
	1.0	−11 (42)		−13 (60)
	0.5	−7 (10)	−20 (10)	−10 (30)
	0.25			−15 (4)
	0.125			+3 (6)
3. Growth hormone	4.0	−7 (19)		−17 (9)
	3.0	−6 (20)	−4 (20)	−13 (20)
	2.5			+4 (4)
	2.0	−5 (10)		
	1.0	−1 (21)		−9 (55)
	0.5			−4 (16)
4. Thyrotropic hormone	3.0	−15 (20)	−33 (10)	−20 (10)
	2.5	−8 (9)	−27 (9)	−4 (9)
	2.0	−20 (14)		
	1.0	−13 (80)	−16 (44)	−10 (72)
	0.5	−10 (24)	−19 (10)	−16 (24)
	0.25			−15 (12)
	0.15			−12 (4)
	0.075			+3 (6)
5. Lactogenic hormone	2.0	−1 (10)	−3 (10)	+11 (10)
	1.0	−7 (9)		−4 (6)
	0.75			+9 (6)
6. Adrenocorticotropic hormone	4.0	+12 (10)	+13 (10)	0 (10)
	2.0	−1 (9)		−16 (9)
7. Gonadotropins: ICSH	1.0	−8 (20)	0 (20)	−7 (20)
FSH + ICSH; each	1.0	+2 (9)		−3 (6)
8. Thyroxin	0.1	−11 (20)	−10 (20)	−6 (20)
	0.02	−3 (10)	−5 (10)	−5 (10)

* Figures in parentheses denote the number of treated animals at each dose level.

Yet growth hormone and ICSH were found not to cause significant effects on blood nonprotein nitrogen and urea even in such operated rats, thus confirming previous results in normal rats (table 5, 2). In contradistinction to the pro-

nounced increase in blood urea, the blood amino acid concentration was found to be slightly lowered by hypophysectomy; also the action of the various hormones appeared to be similar to that in normal rats, thyrotropic lowering it more markedly than growth hormone, and ICSH having no effect (table 5, 2).

In a number of experiments other constituents of the nonprotein nitrogen were also determined. Of these uric acid, total creatinine and glutathione (thiol) were found either lowered or unaffected by thyrotropic hormone. Both higher and lower polypeptides were generally decreased by the hormone (table 2).

Mechanism. It can thus be regarded as established that the thyrotropic but not the growth hormone causes rapid decreases in blood nonprotein nitrogen

TABLE 5

Summary of the effects of hormones on blood nonprotein nitrogen constituents of operated rats*

HORMONE PREPARATION		CHANGE, COMPARED WITH RESPECTIVE CONTROLS†		
Type of hormone	Dose	NPN	Urea N	Amino acid N
	mgm.	*per cent*	*per cent*	*per cent*
1. Thyroidectomized				
Thyrotropic hormone........ {	2.0	−5 (10, ns)	−3 (10, ns)	−9 (10, hs)
	1.0	−10 (19, hs)	−4 (17, ns)	−10 (20, s)
Thyroxin....................	0.2	−4 (4, ns)	−8 (4, ns)	0 (4)
2. Hypophysectomized				
	1.0‡	−17 (10, s)	−10 (10, ns)	−9 (10, ns)
Thyrotropic hormone........ {	1.0	−35 (22, hs)	−43 (22, hs)	−9 (22, s)
	0.2	−5 (7, ns)	0 (7)	−4 (7, ns)
Growth hormone.............	1.0	+11 (9, ns)	−9 (9, ns)	−10 (6, s)
ICSH......................	1.0	−7 (7, ns)	−17 (7, ns)	−1 (5, ns)
Thyroxin*..................	0.25	−54 (7, hs)	−73 (7, hs)	+7 (7, ns)

* Only completely operated animals were included in the tables. All rats were injected 4 hours before the collecting of blood samples, except the group receiving thyroxin, marked by an asterisk, which was injected 24 hours before autopsy.

† Figures in parentheses denote the number of injected animals, the letters statistical significance.

‡ The rats in this group were used two days following the operation, all other groups were from 2 to 7 weeks postoperative.

and urea, while both hormones lower the amino acid concentration of the blood.[7] It has furthermore been shown that thyroxin has a similar action on blood urea as the thyrotropic hormone. The lack of a pronounced effect on blood urea if thyrotropic hormone was given to thyroidectomized rats as well as the effectiveness of thyroxin suggest that this action of the pituitary is mediated by the thyroid. However, a comparison of the blood urea of untreated rats of various types suggests that this may not be the only mode of action of the pituitary

[7] Paschkis (26, 27) suggested that the growth hormone was probably not identical with the "protein metabolism hormone." We were unable to confirm his claim that the latter hormone can be demonstrated in the urine after a high protein diet.

hormone (table 6). If blood urea were decreased only by the thyroid hormone, thyroidectomized rats would be expected to have higher blood urea levels than hypophysectomized rats. Actually, the blood urea level increases much more strikingly after hypophysectomy than following removal of the thyroid. Even more surprising is the pronounced and rapid urea-lowering effect of thyrotropic hormone in hypophysectomized rats, without evident stimulation of their atrophic thyroids. These findings suggest another possible interpretation, namely, that both the thyroid hormone and thyrotropic hormone directly lower blood urea. If that is so, the blood urea of thyroidectomized rats should still be comparatively well controlled through the action of the thyrotropic hormone which is probably secreted at an increased rate in the absence of the thyroid. On the other hand, the hypophysectomized rat, deprived of both the tropic and most of the thyroid hormone would be expected to show high blood urea levels, which can be effectively and rapidly lowered by administration of either of these hor-

TABLE 6

Concentration of blood nonprotein nitrogen constituents of uninjected rats

TYPE OF RAT	NUMBER OF RATS	NPN		UREA-N		AMINO ACID N	
		Group average	Total average	Group average	Total average	Group average	Total average
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Thyroidectomized	10	42.5		20.6		7.3	
	8	42.7	41.4	20.7	21.2	8.5	7.5
	10	39.0		22.2		6.8	
Hypophysectomized	7	85.6		58.4		7.7	
	11	78.6	69.8	52.7	46.5	6.8	7.2
	8	45.2		28.4		7.1	
Normal	ca. 232		38.9		15.8		8.9

mones. It is hoped that this hypothesis may be tested by the use of doubly operated (hypophysectomized-thyroidectomized) rats.

For the mechanism of the described hormonal action on the blood-urea level, the following possibilities have to be considered: a, an action on the kidney, increasing the rate of excretion of urea; b, a change in the distribution of the body fluids, leading to hemodilution, and c, a decrease in the rate of production of urea.

Several experiments were performed to elucidate this question. a. Nitrogen excretion was determined upon several occasions. In one experiment, two individual 24-hour urine samples were collected from rats which were treated with thyrotropic hormone or thyroxin for two days preceding the taking of blood samples[8] (table 1, 5). It was found that thyrotropic hormone caused some retention of nitrogen when compared with uninjected controls, while thyroxin had no effect. In another experiment with hypophysectomized rats, the urine

[8] This experiment was performed in co-operation with Dr. W. Marx.

was collected during the four hour period of hormone treatment. While blood and tissue urea were lowered by 25 per cent by administered thyrotropic hormone, the nitrogen excretion was similar in injected and untreated rats. These findings clearly indicate that the lowered nonprotein-nitrogen levels produced by thyrotropic hormone and thyroxin cannot be attributed to an increased excretion of urea.

b. In a number of experiments nonprotein-nitrogen constituents of muscle and liver were determined, besides those of the blood. It was then found that thyroxin lowered tissue nonprotein nitrogen and in particular urea of hypophysectomized rats almost as much as the corresponding blood levels. Thyrotropic hormone, at the dose employed, affected liver and muscle values of hypophysectomized rats similarly but in normal rats it produced only small decreases in tissue nonprotein nitrogen, urea and amino acids (table 3). Growth hormone showed no appreciable effect on any of these levels. The above findings indicate that the action of the thyroid on blood urea is accompanied by a similar action on tissue urea; it does not therefore appear probable that the observed blood effects can be due to a shift of water from the tissues into the bloodstream. To test for this possibility, however, blood hemoglobin and serum proteins were included in the determinations in a number of experiments (table 2). Serum-protein nitrogen was regularly found to be close to 1.1 per cent in injected as well as in control groups. Hemoglobin content was found quite variable within each group, so that the observed increases of 12 and 35 per cent in the averages of the thyrotropic-hormone treated rats above those of the controls are probably coincidental. In any case, hemodilution must be excluded as a possible explanation of the observed hormone action.

c. A decrease in the rate of production of urea therefore seems the only probable explanation of the observed facts. In that case, the action of the hormone would be through the liver. Actually, thyroid feeding has been shown to increase the d-amino acid oxidase content of liver (28), and both thyrotropic hormone and thyroxin were found to increase liver weights (11, 29). If it is assumed that the increase in d-amino acid oxidase content is paralleled by an increase in l-amino acid oxidase, this would tend to suggest a greater rate of amino acid breakdown and urea formation. But it is possible that it actually only indicates a high rate of protein metabolism, anabolic and catabolic, with rapid transaminations rather than deaminations. However, as in so many other cases, when the action of thyroxin is studied, the dose may be of primary importance; thus, the observed effect may be characteristic only of the hyperthyroid state which is well known to lead to rapid protein catabolism. On the other hand, the effects on blood urea observed in the present study were produced in animals which were definitely not hyperthyroid; they suggest the necessity of further studies of various enzyme systems under similar conditions. Besides possible effects on amino-acid oxidases, changes in the concentration of enzymes more closely associated with the formation of urea might be searched for. For such reasons, liver-arginase determinations have been undertaken (30). The explanation of the mechanism of the action of thyroxin on blood urea has to be postponed until these and similar studies have been completed.

SUMMARY

1. Purified pituitary hormones were investigated regarding their ability to decrease blood and tissue nonprotein-nitrogen constituents within a four hour test period.

2. Urea, the main component of the blood nonprotein nitrogen, was found to be decreased only by thyrotropic hormone and not by growth hormone, as is generally believed.

3. Both thyrotropic and growth hormones were found to lower blood amino acids, the former more effectively than the latter.

4. The action of thyrotropic hormone on blood and tissue urea could be reproduced also by the administration of thyroxin and thus appears to be mediated by the thyroid gland. The action of thyrotropic hormone on blood aminoacids, on the other hand, was not given by thyroxin.

5. In thyroidectomized animals thyrotropic hormone was still effective in lowering blood amino acids, while its action on blood urea was doubtful.

6. The high blood and tissue nonprotein nitrogen and urea of hypophysectomized animals was lowered strikingly by both thyrotropic hormone and thyroxin, the former but not the latter also affecting blood amino acids.

7. These findings were tentatively interpreted as indicating that thyrotropic hormone lowered blood urea directly as well as through stimulation of the thyroid gland, while controlling the amino acid level only by a direct action.

8. Evidence was adduced showing that the observed decrease in blood urea was not due to increased excretion, nor to hemodilution, and therefore appeared to be caused by a decreased production of urea in the liver.

REFERENCES

(1) TEEL, H. M. AND O. WATKINS. This Journal 89: 662, 1929.
(2) TEEL, H. M. AND H. CUSHING. Endocrinology 14: 157, 1930.
(3) GAEBLER, O. H. J. Exper. Med. 57: 349, 1933.
(4) GAEBLER, O. H. AND P. BARTLETT. J. Biol. Chem. 129: 559, 1939.
(5) GAEBLER, O. H. AND H. W. GALBRAITH. Endocrinology 28: 171, 1941.
(6) LEE, M. O. AND N. K. SCHAFER. J. Nutrition 7: 337, 1934.
(7) SCHAFER, N. K. AND M. O. LEE. J. Biol. Chem. 108: 355, 1935.
(8) LEE, M. O. AND G. B. AYRES. Endrocrinology 20: 489, 1936.
(9) LEE, M. O. The pituitary gland. Assoc. for Res. in Nerv. and Mental Diseases 17: 193, 1938.
(10) MARX, W. In press. This Journal.
(11) FRAENKEL-CONRAT, H., M. E. SIMPSON AND H. M. EVANS. This Journal 135: 398, 1942.
(12) FRAENKEL-CONRAT, H., D. L. MEAMBER, M. E. SIMPSON AND H. M. EVANS. Endocrinology 27: 605, 1940.
(13) MARX, W. Unpublished results.
(14) EVANS, H. M., M. E. SIMPSON AND R. I. PENCHARZ. Endrocrinology 25: 175, 1939.
(15) MARX, W. Proc. Soc. Exper. Biol. and Med. 49: 594, 1942.
(16) FRAENKEL-CONRAT, J., H. FRAENKEL-CONRAT, M. E. SIMPSON AND H. M. EVANS. J. Biol. Chem. 135: 199, 1940.
(17) FOLIN, O. AND H. WU. J. Biol. Chem. 38: 81, 1919.
(18) FOLIN, O. J. Biol. Chem. 51: 377, 1922.
(19) FOLIN, O. AND A. SVEDBERG. J. Biol. Chem. 88: 77, 1930.
(20) ROSE, W. C., D. M. HELMER AND A. CHANUTIN. J. Biol. Chem. 75: 543, 1927.

(21) FOLIN, O.   J. Biol. Chem. **101:** 111, 1933; **106:** 311, 1934.
(22) SNELL, F. D.   Colorimetric methods of analysis.   D. van Nostrand Co., N. Y., 1937, P. 323.
(23) SANFORD, A. H., C. SHEARD AND A. E. OSTERBERG.   Am. J. Clin. Pathol. **3:** 405, 1933.
(24) FRAENKEL-CONRAT, H., V. V. HERRING AND H. M. EVANS.   Endocrinology **30:** 485, 1942.
(25) REINHARDT, W. O.   Proc. Soc. Exper. Biol. and Med. **50:** 81, 1942.
(26) PASCHKIS, K. E.   Endocrinology **23:** 368, 1938.
(27) PASCHKIS, K. E. AND A. SCHWONER.   Endocrinology **26:** 117, 1940.
(28) KLEIN, J. R.   J. Biol. Chem. **131:** 139, 1939.
(29) STERNHEIMER, R.   Endocrinology **25:** 899, 1939.
(30) FRAENKEL-CONRAT, H. AND H. M. EVANS.   Science **95:** 305, 1942.

UTILIZATION BY THE RAT OF VITAMIN A ADMINISTERED PERORALLY AND INTRAMUSCULARLY

O. W. BARLOW AND HUGO KOCHER

From the Research Laboratories, Winthrop Chemical Company, Inc., Rensselaer, N. Y.

According to Funk (1) vitamin A was observed to be biologically inactive unless administered by way of the gastrointestinal tract. Wollman and Vagliano (2), on the other hand, reported that cod liver oil of unstated potency was curative when given to vitamin A deficient rats whether administered orally, subcutaneously or intraperitoneally. However, these data did not permit a satisfactory comparison of the relative effectiveness of such therapy by the routes of administration tested due to severe local intolerance to the parenteral injections, with a high resultant mortality.

Blegvad (3) materially ameliorated the symptoms of a severe ophthalmia in man by means of two subcutaneous injections of a 10 per cent olive oil solution of a potent cod liver oil concentrate: six such injections gave complete symptomatic relief. Bloch (4) was able to relieve the ophthalmia of a nine-year old boy by means of intramuscular injections of oil containing vitamin A.

Koehne and Mendel (5) reported that vitamin A was utilized by the rat following parenteral administration of potent oils. The degree of utilization noted was better after subcutaneous than after intraperitoneal injections, but utilization after oral medication was quite superior to that noted after either parenteral route. Leak (6) also noted that clinical improvement was usually observed in suitable cases following the parenteral injection of a concentrate containing vitamins A and D.

Isaacs, Jung and Ivy (7) observed no difference in the degree of utilization of vitamin A by normal adults or by persons in whom an attempt was made to produce a vitamin A deficiency by dietary measures, whether medication with vitamin A was by the oral or intramuscular routes. The subjects were tested by means of the Hecht adaptometer and the biophotometer.

Recently, Lease, Lease, Steenbock and Baumann (8) reported studies of the comparative values of injected carotene and vitamin A. They conclude that intraperitoneal or subcutaneous injections of aqueous colloidal suspensions or oily solutions of carotene are effective in the treatment of A-avitaminotic rats, but that the amounts needed are from 10 to 100 times as great as when the carotene is given orally. Hepatic storage of vitamin A was found to be very poor following parenteral injections, since carotene was deposited in various sites, and was only partially available to the animal. Much of the injected carotene could not be traced.

By contrast vitamin A given parenterally in colloidal aqueous suspension in weekly dosages of 30 to 750 Blue units was found to be as effective as when given orally. Oily solutions were more effective orally.

Preliminary experiments in this laboratory indicated, on the basis of the official (U.S.P.) biological assays, that the curative effects of oil dilutions of vitamin A concentrates injected intramuscularly were quite inferior to the same dosages given orally. The lack of any concise information of quantitative value in the literature as to the comparative degrees of utilization of vitamin A when given by the oral and parenteral routes suggested the need of such data. Accordingly, this paper presents the results of parallel U.S.P. biological assays after the oral or intramuscular administration of standard doses of the following preparations of vitamin A: 1, a vitamin A concentrate in sesame oil; 2, the U.S.P. vitamin A reference oil no. 2, and 3, vitamin A concentrates in propylene glycol.

METHODS. The assay procedures duplicated in all respects those described for biological assays of vitamin A in the U. S. Pharmacopoeia XI, Second Supplement. However, in one series of animals the reference oil was administered as a weekly (rather than daily) oral dose in order that a direct basis for comparison with weekly intramuscular doses would be available. The oral supplements were of such concentration that the daily dosage was contained in a volume of 0.1 cc. When the fraction was given once weekly it was contained in 0.2 cc. Exceptionally, due to poor nutrition, the voluntary oral intake of the vitamin supplement was either slow or unsatisfactory. Under such conditions the supplement was given by stomach tube until such time as the animals would consume it voluntarily.

Intramuscular injections in oil were made into the belly of the gluteal muscle. The weekly doses were given on alternate sides in 0.1 cc. In biweekly injections alternate sides were also used, and a volume of 0.05 cc. was given by means of a special 0.25 cc. syringe.

The propylene glycol supplements were given in the following volumes: daily oral, in 0.05 to 0.025 cc.; weekly intramuscular, in 0.1 to 0.05 cc.; single intramuscular, in 0.1 cc. The smaller volumes were preferable in that any local intolerance was minimized and oral supplements were more readily consumed. In addition to the usual criterion of weight gain, careful attention was given to the possible presence of ophthalmia and its severity. Readings were made at the beginning and at intervals during the assay period. The numerical data are presented in table 1.

DISCUSSION. It is evident that vitamin A in oil either in the form of a concentrate, or in the form of cod liver oil (reference oil), when administered intramuscularly, is only from 10 to 15 per cent as effective as when given orally. Weekly oral administration, as would be expected, is not as effective as the same vitamin supplement divided into daily oral dosages. Subdivision of the weekly intramuscular supplements into biweekly dosages increases the effectiveness noticeably.

On the other hand, when vitamin A is administered intramuscularly in propylene glycol it appears to be at least as effective as when given orally in the same menstruum. Even when the entire medication for the 28-day period is given as a single dose, the weight gain corresponds approximately to that noted for the control daily oral dosage series. This indicates that this solvent in contrast to

TABLE 1

Effect of various modes of administration of vitamin A on the growth and eye condition of rats

| PRODUCT | MODE OF ADMINISTRATION | | DOSE, U.S.P. UNITS | NUMBER OF RATS (INIT-IALLY) | PER CENT MORTAL-ITY | PER CENT DIS-CARDED§ | EYE CONDITION¶ | | AVERAGE GAIN |
	Route	Interval					Start‖	Finish‖	
									gms.
Vitamin A in se-same oil, 50,000 units per cc.* (January)	Oral	Daily	2	11	0	27	0.94	0.20	17.2
	Oral	Daily	3	11	9	9	0.30	0.0	27.6
	Intram.	Weekly	1	12	58	0	0.0	1.3	−21
	Intram.	Weekly	2	12	42	0	0.7	1.14	−19
	Intram.	Weekly	4	12	50	0	1.25	2.16	− 2
USP reference oil no. 1 (Febru-ary)	Oral	Daily	1	10	10	0	0.50	0.44	21.7
	Oral	Daily	2	10	10	0	1.75	0.65	49.4
	Oral	Daily	3	10	0	0	1.40	0.05	74.3
	Oral	Weekly	2	10	0	20	0.37	0.0	40.5
	Intram.	Weekly	4	10	40	0	0.60	0.90	13.1
	Intram.	Weekly	8	· 10	20	0	0.12	0.62	20.6
	Intram.	Weekly	12	10	30	0	0.14	0.0	24.9
	Intram.	Biweekly	12	10	20	0	0.25	0.0	31.3
Vitamin A in pro-pylene glycol 80,000 units per cc.† (July)	Oral	Daily	2	12	16.5	16.5	1.48	1.62	17.1
	Intram.	Single dose	2	12	16.5	25	1.86	1.40	16.0
	Intram.	Single dose	4	12	8	25	1.12	0.12	30.2
	Intram.	Single dose	8	9	0	0	1.00	0.40	43.2
Same as above, refrigerated 5 weeks (August)	Oral	Daily	1	11	18	9	0.12	0.37	17.8
	Intram.	Weekly	1	12	16.5	0	0.25	0.0	22.8
	Intram.	Weekly	2	12	0	0	0.75	0.50	30.1
	Intram.	Weekly	3	9	22	0	1.30	0.43	33.7
Vitamins A and D in propylene glycol‡ (August)	Oral	Daily	2	11	18	0	0.77	0.50	26.0
	Intram.	Weekly	2	11	0	0	0.77	0.09	48.8
	Intram.	Weekly	3	7	0	0	1.07	0.0	53.1
USP reference oil no. 2 (August)	Oral	Daily	1	10	10	0	0.2	0.1	17.9
	Oral	Daily	2	12	8.5	0	0.45	0.2	29.3
	Oral	Daily	3	10	0	0	0.10	0.15	45.2

* A molecular distillate of vitamin A (biologically standardized). Diluted with sesame oil from 200,000 to 50,000 units per cc.

† Prepared from an alcohol-soluble vitamin A concentrate of high potency. Stability of vitamin A satisfactory after $6\frac{1}{2}$ months of storage at 37.5° C.

‡ Prepared as above: 24,800 units of viatmin A per cc. with 2,000 USP units of vitamin D_2 as crystalline calciferol added.

§ Animals that gained less than 25 per cent of the entire group average were not included in the calculation.

¶ Rated according to the standard scheme used by the U. S. Pharmacopoeia Advisory Board. The figures are averages for only those animals not discarded because of death or poor weight gain.

‖ Of test.

oil permits a satisfactory absorption from the muscle, and may be attributed to the fact that propylene glycol is readily miscible with the tissue fluids, whereas oil remains in depots for considerable periods of time and probably permits destruction of vitamin A at the site of injection. Weekly intramuscular injections in propylene glycol are even more effective than the same dosages given in daily portions orally, and responses from such medication compare favorably with the same number of units (in oil) given as daily supplements.

The data on the anti-xerophthalmic effects of medication of the animals support the conclusions as to relative effectiveness drawn from the data on body weights, but permit a clear cut differentiation as to differences in absorption rates with the different solvents.

These data in general confirm and extend those previously reported, but permit a quantitative comparison by U.S.P. methods (or necessary modifications) of the effects of oral and intramuscular routes of administration of vitamin A in oil and in propylene glycol. We are in agreement with Lease et al. that unit growth responses require much larger amounts of oil solutions of vitamin A when given by the parenteral route, than are required in oral administration. When oily solutions of vitamin A are injected intramuscularly, large amounts of the oil and solute remain *in situ*. Utilization of the vitamin is poor partly because of lack of absorption, but also because of destruction at the site. The curative effects of injections of vitamin A in propylene glycol would seem to be qualitatively comparable with those of Lease et al. after injections of colloidal aqueous suspensions.

Our data indicate that solutions of vitamin A in propylene glycol are utilized equally well whether given intramuscularly or orally. Utilization of the usual weekly dose was equally satisfactory whether given as a single injection or at biweekly intervals. These studies suggest that stable propylene glycol solutions of vitamin A may offer an additional approach to the clinical problem of therapy of acute vitamin A deficiencies.

SUMMARY

Vitamin A administered intramuscularly in oil to vitamin A deficient rats is approximately 10 to 15 per cent as effective as the same material given orally.

On the other hand, solutions of vitamin A in propylene glycol are at least as effective by the intramuscular route as when given orally, even when the oral supplements are given in a larger number of doses. The effectiveness of intramuscular injections, whether in oil or in propylene glycol, increases with the division of the dose.

REFERENCES

(1) Funk, C. The vitamins. 2nd ed. Williams & Wilkins Co., Baltimore, Md. 1922.
(2) Wollman, E. and M. Vagliano. Compt. rend. soc. de biol. 88: 336, 1923.
(3) Blegvad, O. Ugeskr. f. Laeger 85: 942, 1923.
(4) Bloch, C. E. Ugeskr. f. Laeger 87: 1183, 1925.
(5) Koehne, M. and L. B. Mendel. J. Nutrition 1: 399, 1929.
(6) Leak, W. N. Lancet, Part I: 599, 1938.
(7) Isaacs, B. L., F. T. Jung and A. C. Ivy. Arch. Ophthalmol. 24: 698, 1940.
(8) Lease, J. G., E. F. Lease, H. Steenbock and C. A. Baumann. J. Lab. and Clin. Med. 27: 502, 1942.

THE BULBAR PROJECTION OF THE TRIGEMINAL NERVE[1]

W. A. McKINLEY AND H. W. MAGOUN

From the Institute of Neurology, Northwestern University Medical School, Chicago

Within recent years the introduction of bulbar tractotomy for the relief of trigeminal neuralgia (Sjöqvist, 1938) has stimulated interest in the detailed arrangement of trigeminal connections within the medulla oblongata. Degeneration studies after differential section of the sensory root in animals (Davis and Haven, 1933) and clinical observations in cases of posterior inferior cerebellar artery occlusion (Smyth, 1939) have given support to the view that the bulbar representation of the trigeminal nerve is a divisional one, i.e., the fibers from the three divisions of the nerve run separately down the spinal fifth tract. Other observers (Déjérine, 1914) have postulated an "onion peel" representation, in terms of concentric zones of the face extending outward from the peri-oral region.

It appeared that evidence for one or the other of these conflicting views might be obtained by resort to a technique not heretofore employed in this connection, and in the present study the bulbar projection of the trigeminal nerve has been studied by examining the distribution of action potentials evoked by single shock stimuli applied to peripheral trigeminal branches and recorded centrally with the cathode ray oscillograph.

METHODS. In a series of cats, anesthetized with nembutal or decerebrated under ether, the supraoptic branch of the ophthalmic nerve, one or more fascicles of the infraorbital branch of the maxillary nerve, and the anterior mental branches of the mandibular nerve, were routinely employed for stimulation at the face. The stimuli consisted of single condenser discharges delivered through a transformer.

The action potentials evoked were recorded from the interior of the medulla with a steel needle electrode, insulated except for 0.1 to 0.2 mm. at the tip, and oriented within the brain with the Horsley-Clarke instrument in the manner described by Ranson (1934). An indifferent electrode was placed on the frame of the instrument or in the cerebellar cortex. The potentials were amplified with a resistance-capacity coupled differential amplifier and visualized with a cathode ray tube. The stimulus was synchronized with the sweep of the oscillograph so that a single sweep and a single shock could be produced with a manual switch. Exploration was undertaken in a systematic fashion and in each experiment the points of recording were subsequently determined by microscopic examination of Weil or cresyl violet stained sections cut serially by the frozen-section method of Marshall.

I. CHARACTERISTICS OF RECORDED POTENTIALS. *Potentials from the sensory trigeminal root and the spinal fifth tract.* When a single supramaximal shock is

applied to a group of trigeminal fibers at the face, and the tip of the recording electrode is in contact with the central processes of these fibers in their intramedullary course within the sensory root or the spinal fifth tract, the unvarying sign of their activity is the fast positive spike seen in figure 1, 1, 3A, 4A. This spike commences after a latency of about 1 msec., and both peripheral and central measurements indicate that it signalizes the discharge of fibers conducting at a rate between 40 and 50 meters per sec. This rapid conduction rate is in keeping with the low threshold of peripheral stimulation required for its elicitation, which is that of mammalian A fibers. When two stimuli to the same nerve are employed, the second primary afferent spike is conducted unattenuated at an interval of 3 msec. (fig. 1, 3B). It is relatively resistant to anoxia (fig. 1, 4A–D).
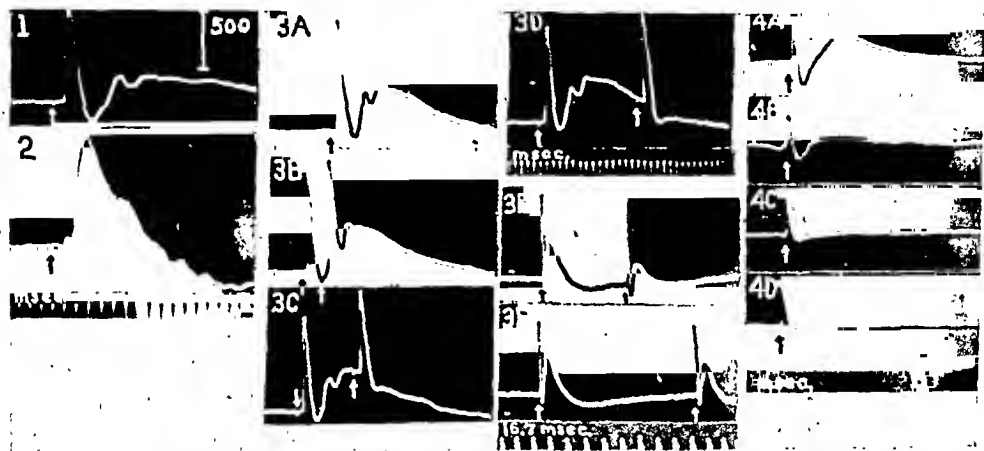
Fig. 1. Action potentials recorded from the spinal fifth tract (1, 3, 4) and spinal fifth nucleus (2) upon stimulation of an infraorbital branch of the trigeminal nerve at the face. The application of supramaximal shocks is indicated by arrows. In 3 B–F, the intervals between shocks are 3, 8.5, 15, 81 and 145 msec. Records 4 B–D were taken 40, 90 and 120 seconds after stabbing the heart. Amplification in 4 from another experiment is 3 times that of 1, 2 and 3. In all records an upward deflection indicates positivity at the needle electrode.

The initial positive spike is followed by a more variable later positive wave (fig. 1, 1, 3A, 4A), which a number of characteristics suggest to be the result of a potential change within the adjacent spinal fifth nucleus, rather than a sign of activity of slower conducting primary afferent neurons within the tract. This later positive wave is more pronounced when the recording tip of the electrode is located medially in the tract, near the spinal fifth nucleus. It has not been encountered when leading from the intramedullary course of the sensory root anterior to the spinal fifth nucleus. The threshold of peripheral stimulation for producing the second wave is the same as that for the initial fast spike, whereas if slower conducting fibers were concerned it might be expected to be higher. When tested with two stimuli, the excitability cycle of the neural elements responsible for the late positive wave is found to be remarkably longer than that of those whose activity causes the initial fast spike (fig. 1, 3A–F). The second wave is completely absent at testing intervals up to 15 msec. (fig. 1, 3B, C and D)

and its relatively unresponsive period persists for 160 msec. (fig. 1, 3E and F). In other experiments the absolutely unresponsive period has ranged between 10 and 20 msec. and the relatively unresponsive period between 70 and 160 msec. The late positive wave is quite sensitive to anoxia and figure 1, 4A–D shows its disappearance at successive intervals after stabbing the heart, while the initial spike remains unaffected. If slower conducting primary afferent fibers were concerned here, it might be expected that they would be no more susceptible to anoxia than fast conducting fibers, or indeed less so. In other experiments the time course of depression of the late wave with anoxia, induced by breathing nitrogen, was found to be similar to that of potentials derived from the spinal fifth nucleus; and full recovery upon again breathing air was subsequently encountered.

Potentials from the spinal fifth nucleus. With the recording electrode in the nucleus of the spinal fifth tract, stimulation of a trigeminal branch at the face leads to a discharge of secondary trigeminal neurons signalized by a large positive deflection commencing 0.5 to 1 msec. later than the discharge of primary neurons in the spinal fifth tract (fig. 1, 2; fig. 2, 2A). The synaptic delay in the instances shown amounted to 0.8 and 0.9 msec., respectively. Almost invariably the nuclear discharge is preceded by a small positive notch (fig. 1, 2; fig. 2, 2A–D), which coincides temporally with the discharge of primary neurons within the tract. Whether this is recorded directly from the terminals of primary fibers ramifying within the nucleus, or as a result of "electro-tonic spread" from the large concentration of primary fibers firing in the nearby spinal fifth tract, cannot be said. The fact that this primary notch is present in records taken from the reticular formation medial to the nucleus of the spinal fifth tract (fig. 2, 3A–E) appears to favor the latter possibility.

The constant aspect of the configuration of the nuclear spike is the fast rising phase which attains a peak about 1 msec. after its commencement, and reflects fairly closely the period of discharge of primary neurons in the spinal fifth tract. The descending limb of the nuclear spike is always prolonged beyond the period of discharge of the primary fibers, however (fig. 1, 1 and 2; fig. 2, 1B and 2A), and the duration of the descending limb exhibits great variability. Nuclear spikes of all durations between 2.5 and 17 msec. have been observed. At durations of 2.5 msec., the nuclear wave is spike-like and suggests the synchronous discharge of a group of similar secondary neurons. Waves of longer duration may be attributed either to the discharge of additional neurons or to the repetitive firing of those responsible for the early part of the wave.

As might be expected the recovery times of different nuclear spikes are also variable. The early phase of the excitability cycle of a nuclear spike of rather short duration is shown in figure 2, 2B–D. It is seen that the secondary neurons are relatively unresponsive after activity for a far longer time than are the primary afferent neurons (fig. 2, 1B). In different preparations the absolutely unresponsive periods of elements responsible for the nuclear spikes range between 3 and 6 msec., and the relatively unresponsive states persist for from 30 to 170 msec.

Records taken from the adjacent reticular formation, medial to the nucleus of the spinal fifth tract and in the region of passage of axons emerging from it, are similar to those from the nucleus itself. The discharge shown in figure 2, 3A, recorded from the reticular formation 1 mm. from the edge of the spinal fifth nucleus, is remarkable for its prolonged duration. A series of records, showing the effect of a testing volley following a conditioning volley at increasing intervals (fig. 2, 3B–E), demonstrate that the recovery time for the elements producing the delayed activity is different from that of those responsible for the initial phase of the wave, which presumably represents the discharge of axons of the secondary trigeminal neurons. It is suggested that the delayed discharge is the result of the activation of interneurons, and such presumed indications of internuncial activity have been encountered from within and close to the spinal
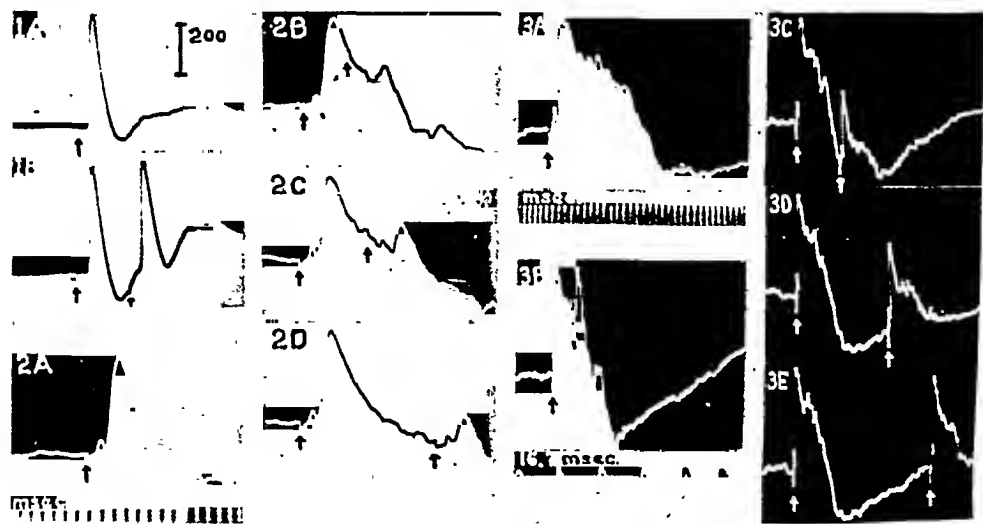
Fig. 2. Action potentials recorded from the spinal fifth tract (1), spinal fifth nucleus (2), and reticular formation 1 mm. medial to spinal fifth nucleus (3), upon stimulation of an infraorbital branch of the trigeminal nerve at the face. Intervals between shocks: in 1 B, 4 msec.; in 2 B–D, 3.7, 5.8 and 11.3 msec.; in 3 B–E, 7.8, 17, 34 and 57 msec. Amplification same in all.

fifth nucleus in many experiments. Although no detailed analysis has been made to clarify its derivation, the presence of a later negative deflection following the nuclear spike should be noted (fig. 2).

II. INTRAMEDULLARY REPRESENTATION OF THE TRIGEMINAL NERVE. *Spinal fifth tract.* In their course in the sensory root and spinal fifth tract the primary afferent fibers of the three trigeminal divisions are laminated in a dorso-ventral order which is the inverse of their peripheral distribution in the face, as is shown in figure 3. In this instance, the needle electrode was inserted into the rostral part of the left spinal fifth tract and halted at half millimeter intervals 1 to 7. At each stop records were taken of the electrical activity induced by serially stimulating peripheral branches of the mandibular, maxillary and ophthalmic divisions of the trigeminal nerve. It can be seen that fast positive spikes from fibers of the mandibular division, shown by squares, were obtained at stops 2

and 3 in the dorsal part of the spinal fifth tract (fig. 3). Those from the maxillary division, shown by circles, were found at stops 4, 5 and 6, chiefly 4 and 5, in the intermediate part of the tract, and those from the ophthalmic division, shown by triangles, from stops 6 and 7 in the ventral part of the tract (fig. 3). The results demonstrate that the representation of the trigeminal nerve within the spinal fifth tract is by divisions, and the arrangement of the divisions is the inverse of their peripheral distribution, the mandibular division being situated
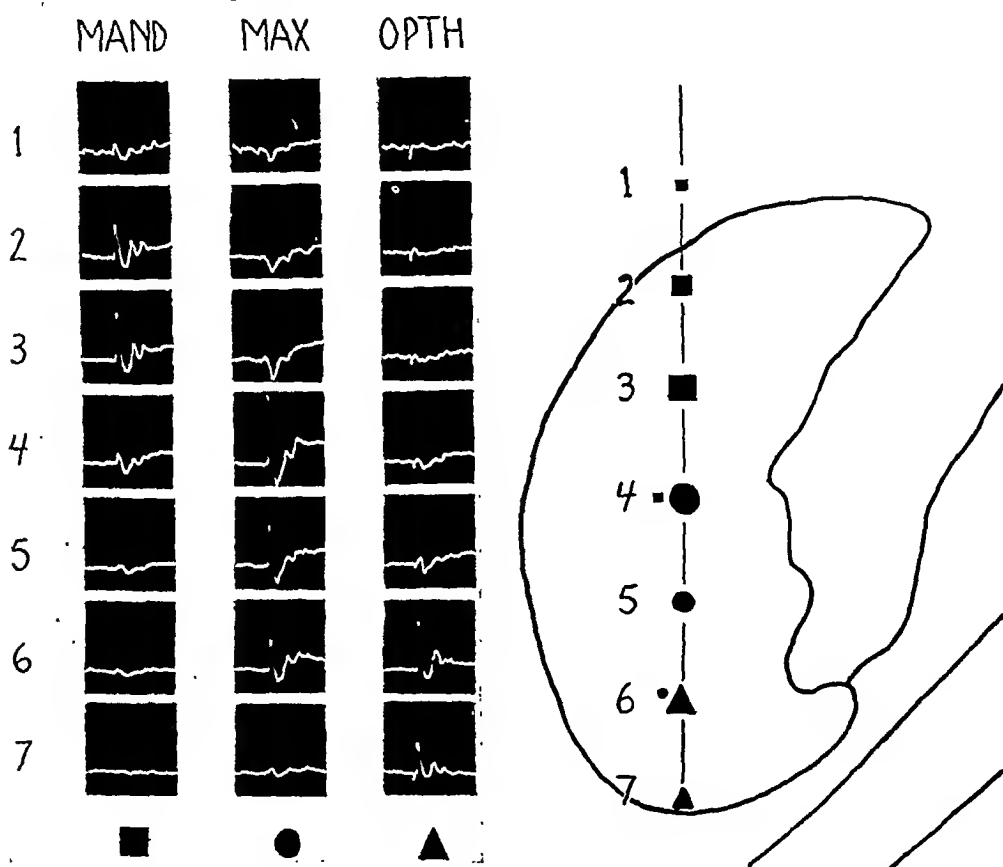
Fig. 3. Electrode lowered into left spinal fifth tract and halted at half millimeter intervals 1 to 7. Records of potentials induced by serially stimulating branches of the three trigeminal divisions at each stop are shown.

dorsally, the maxillary division in an intermediate position, and the ophthalmic division ventrally in the spinal fifth tract.

Further data are shown in figure 4 on drawings of transverse sections of the medulla, upon which are indicated the results of a number of experiments. With the trigeminal divisions designated as in figure 3, points from which primary spikes were recorded are shown with stippled symbols and those yielding spikes from secondary neurons, with solid symbols. At the level of figure 4 A, the rotation of the sensory root (Davis and Haven, 1933) is not yet complete. Here the mandibular, maxillary and ophthalmic fibers are respectively in the

dorso-lateral, intermediate and ventro-medial parts of the sensory root. Throughout the main extent of the spinal fifth tract (fig. 4, B, C, D), the dorso-ventral lamination of the three divisions seen in figure 3 is encountered. Spikes from primary afferent fibers of the mandibular division were recorded as far caudally as the level of the obex (fig. 4 E). Maxillary and ophthalmic fibers were detected as far caudally as the first cervical segment (fig. 4 F).

Main sensory and spinal fifth nucleus. Secondary trigeminal spikes recorded from the main sensory nucleus (fig. 4 A) and the nucleus of the spinal fifth
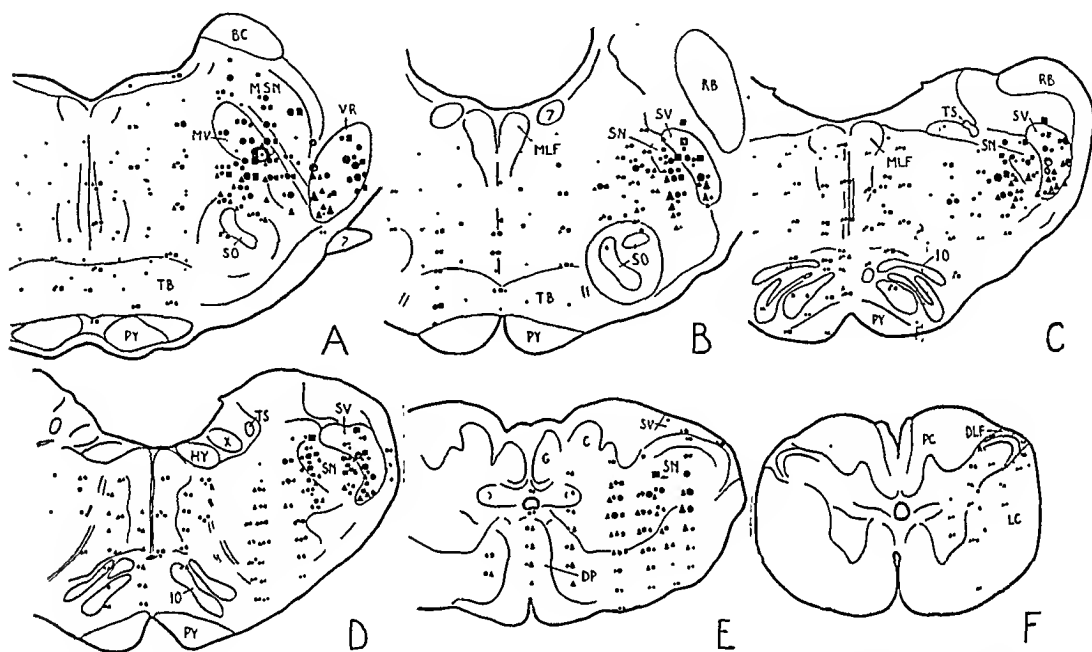
Fig. 4. Six transverse levels through the medulla of the cat showing the distribution of points from which action potentials were recorded upon peripheral trigeminal stimulation. Symbols as in figure 3. Abbreviations are as follows:

BC, brachium conjunctivum; *DLF*, dorsolateral fasciculus; *DP*, decussation of pyramids; *HY*, hypoglossal nucleus; *IO*, inferior olivary nucleus; *LC*, lateral column; *MLF*, medial longitudinal fasciculus; *MNS*, main sensory nucleus of trigeminal; *MV*, motor nucleus of trigeminal; *PC*, posterior column; *PY*, pyramid; *RB*, restiform body; *SO*, superior olivary nucleus; *SN*, nucleus of spinal fifth tract; *SV*, spinal fifth tract; *TB*, trapezoid body; *TS*, tractus solitarius; *VR*, sensory root of trigeminal; *X*, vagal nucleus; *7*, genu of facial nerve.

tract (fig. 4 B–D) were similar in configuration. Throughout the extent of the spinal fifth nucleus a tendency was indicated for a dorso-ventral lamination of the three trigeminal divisions similar to that in the spinal fifth tract, but with greater overlap (fig. 4 B–D). No rostro-caudal projection of trigeminal divisions upon the secondary neurons of the spinal fifth nucleus could be detected.

Secondary trigeminal pathways. Potentials of smaller magnitude, but of about the same latency as the nuclear spikes, were recorded from points distributed through the reticular formation and over into the medial part of the opposite side of the medulla, through its rostro-caudal extent (fig. 4). These are attrib-

uted to the discharge of secondary trigeminal connections, which appear to course through the reticular formation as arcuate fibers and pass to the ventro-medial part of the opposite side of the medulla in considerable numbers. From this region they may be followed forward in the medial lemniscus to the thalamus (Magoun and McKinley, 1942). In this part of their course the connections from the three trigeminal divisions appear to be indiscriminately mixed. Indications of reflex connections with bulbar motor nuclei have also been encountered.

"Onion peel" theory. In other experiments, comparison of the distribution of fibers from two mandibular branches, the anterior mental and the auriculo-temporal, was made as a test for the "onion peel" theory of trigeminal representation within the medulla. Since the areas innervated by these two nerves are respectively at the center and periphery of the "onion," a significant difference in the central distribution of their fibers should be expected. Their course in the spinal fifth tract and their most caudal extent, as detected by this method of study, were, however, identical. In addition, the distribution of secondary neurons in the spinal fifth nucleus which were fired by fibers of these two branches was the same. It is difficult to postulate other than a divisional representation in the face of such findings.

DISCUSSION. The results of these experiments support the view that the representation of the trigeminal nerve in the spinal fifth tract is a divisional one, both as regards an inverted dorso-ventral lamination and as regards a varying rostro-caudal extent. A divisional representation in the secondary neurons of the spinal fifth nucleus is also demonstrated to the extent of a dorso-ventral lamination similar to that in the spinal fifth tract, but with greater overlap. No definite rostro-caudal projection upon the secondary neurons could be detected, however, for secondary spikes were recorded throughout the extent of the spinal fifth nucleus in the case of each trigeminal division.

The only action potentials recorded from the spinal fifth tract and clearly identified with primary afferent fibers in these experiments have been from fast conducting and, presumably, large fibers. The results of Harrison and Corbin (1942) indicate that they mediate the sensation of touch. Potentials from fine and, presumably, slower conducting fibers, which have been shown to descend in the spinal fifth tract by Windle (1926), could not be detected and the distribution of these fine fibers could not be determined.

The bulbar course of trigeminal afferents in the spinal fifth tract and nucleus constitute an anatomical arrangement which is analogous to the connection of dorsal root afferents with sensory neurons of the dorsal gray column of the spinal cord and the potentials recorded from the spinal fifth tract are similar to the afferent cord potentials recorded by Gasser and Graham (1933) and Hughes and Gasser (1934). In both cases there occurs a fast spike followed by a more disperse intermediary wave. The initial deflection is attributed to the discharge of primary afferent fibers, and the later wave to the subsequent firing of adjacent neurons, which in the trigeminal system can be recorded from, in relative isolation, in the nucleus of the spinal fifth tract.

SUMMARY

The character and distribution of action potentials recorded from points within the medulla of the cat upon electrical stimulation of peripheral branches of the three trigeminal divisions are described.

In their course in the sensory root and spinal fifth tract, fast conducting fibers of the three trigeminal divisions are laminated in a dorso-ventral order which is the inverse of their distribution in the face. Fibers of the mandibular division are detected as far caudally as the obex; those of the maxillary and ophthalmic divisions as far caudally as the first cervical segment.

The neurons of the spinal fifth nucleus fired by primary fibers of the three trigeminal divisions are laminated in a dorso-ventral order similar to that of the spinal fifth tract, but with greater overlap. Secondary trigeminal pathways pass diffusely across the reticular formation to the ventro-medial part of the opposite side of the medulla and ascend in relation to the medial lemniscus.

REFERENCES

DAVIS, L. AND H. A. HAVEN. Arch. Neurol. and Psychiat. 29: 1, 1933.
DÉJÉRINE, J. Sèmiologie des Affections du Système Nerveux. p. 838, 1914, Paris.
GASSER, H. S. AND H. T. GRAHAM. This Journal 103: 303, 1933.
HARRISON, F. AND K. B. CORBIN. Anat. Rec. 82: 26, 1942.
HUGHES, J. AND H. S. GASSER. This Journal 108: 307, 1934.
MAGOUN, H. W. AND W. A. McKINLEY. This Journal, in press, 1942.
RANSON, S. W. Psychiat. en neurol. bl. 38: 534, 1934.
SJÖQVIST, O. Acta Psychiat. Kbh., Supplement, 1938.
SMYTH, G. E. Brain 62: 41, 1939.
WINDLE, W. F. J. Comp. Neurol. 40: 229, 1926.

PROLONGED ACTION OF HISTAMINE[1]

CHARLES F. CODE[2] AND RICHARD L. VARCO

From the Departments of Physiology and Surgery, University of Minnesota, Minneapolis

When histamine is placed in the body in watery solution, it is absorbed rapidly and its effects are of relatively short duration (Dale and Laidlaw, 1910). The present investigation was undertaken with the aim of developing a procedure whereby the period of action of single injections of histamine could be extended so that the effects of prolonged, continuous action of histamine might be studied.

EXPERIMENTAL PROCEDURE. The principle of the various procedures used in trying to extend the action of histamine was to mix it with a material which would be absorbed slowly, the aim being to have histamine gradually liberated from the site of injection. Two tests were used to determine the rate of absorption of histamine from the injected mixtures. In the first test, using guinea pigs, a comparison was made between the effects of large doses of histamine in saline solution and similar doses of histamine in one of the mixtures. While this test was satisfactory for the preliminary identification of slowed absorption of histamine, it did not give any measure of the duration and intensity of the prolonged action. The second test allowed a quantitative estimation of the period of action of histamine. In it the amount, acidity and duration of secretion from gastric pouches of dogs were measured in response to the injection of the various mixtures of histamine. The pouches of the stomach were prepared according to the method of Heidenhain with the animal under ether or pentobarbital sodium (nembutal) anesthesia. Sufficient time for complete recovery and healing was allowed before tests were commenced. An overnight fast preceded all tests and no food was given during the period of observation. The volume of gastric juice secreted was measured each hour. The free and total acidity of the hourly samples was determined by titration with tenth-normal sodium hydroxide using Töpfer's reagent and phenolphthalein as indicators. The hydrochloric acid content of each sample of juice was calculated from the free acidity titration values; the total output of hydrochloric acid from the gastric pouch, in grams of hydrochloric acid, was computed for each test. The free acidity was expressed in the usual clinical units of degrees of free acid.

An attempt was first made to prolong the action of histamine by suspending it in mineral oil. Finely powdered histamine was ground with glycol stearate and then suspended in mineral oil. Guinea pigs into which this suspension was injected showed symptoms as quickly and died as frequently as animals

---

[2] Present address, Mayo Foundation, Rochester, Minnesota.

receiving the same doses of histamine in saline solution. Similar results were obtained when particles of histamine were covered with paraffin and suspended in oil. Definite protection was obtained, however, when histamine was placed in a mixture of beeswax and mineral oil.

In preparing the beeswax mixture the salt of histamine was first ground to as fine a powder as possible in an agate mortar. Histamine acid phosphate was used generally although a few experiments were performed with the dihydrochloride. When the dihydrochloride was used, care was taken to dry it thoroughly before mixing it with the beeswax. Weighed quantities of the finely powdered histamine were placed in a hot agate mortar. The mortar had been preheated in water brought to the boil and in addition was sometimes kept warm over a hot water bath. Hot molten beeswax was added from a pipet or a calibrated dropper and the histamine and beeswax were mixed thoroughly. Hot mineral oil then was added and the mixture stirred until of uniform consistency. While still molten the mixture was drawn into 1 cc. tuberculin syringes. These had been warmed by rinsing with hot mineral oil. On cooling to room temperature the mixture formed a semisolid mass which at usual room temperatures was injected easily through 20 or even 22 gauge needles.

The concentration of histamine usually used ranged from 15 to 100 mgm. of the base per cubic centimeter of the mixture. In the case of the acid phosphate this required placing approximately 42 to 278 mgm. in each cubic centimeter of the mixture. Satisfactory preparations with concentrations greater than 100 mgm. of the base per cubic centimeter have been made with the less bulky dihydrochloride salt.

The amount of beeswax in the mixture ranged from a fourth to a fifth of the total volume of liquid employed. Ordinary beeswax, beeswax mixed with a little resin and bleached beeswax have been used in preparing the mixture. All have given about equally satisfactory results. In one experiment, mixtures made with ordinary beeswax and beeswax containing a little resin were compared. The beeswax containing a little resin seemed to give a slightly more tenacious mixture from which absorption may have been a little slower than from the other mixtures. The bleached beeswax gives a white or nearly white mixture.

As a routine, ordinary mineral oil was used as a diluent. The amount employed varied from three-fourths to four-fifths of the total volume of liquid in the mixture. The proportions of the various constituents used in the preparation of the mixture are illustrated in the following typical example: if 500 mgm. of histamine acid phosphate, containing 180 mgm. histamine base, was to be made up in a batch of mixture to contain 100 mgm. histamine base per cubic centimeter, 1.8 cc. of liquid was used of which 0.4 cc. was beeswax and 1.4 cc. mineral oil. The procedure does not take into account the volume of the powered histamine nor does it allow for the shrinkage which occurs when the hot beeswax and mineral oil cool.

Some experiments have been performed using sesame oil in place of mineral oil as a diluent. The mixture with sesame oil is somewhat firmer than with mineral oil and to prevent difficulty when injecting, not more than a fifth of the total volume of the mixture should be beeswax.

The melting point of a number of different batches of the mixture of histamine, beeswax and mineral oil was determined. In making this determination, the mixture was placed in a capillary glass tube or between two cover slips and signs of melting were watched for through a magnifying system as heat was applied through alcohol to the capillary tube and through metal to the cover slips. As determined by these procedures the melting point of the mixture of histamine, beeswax and mineral oil was above body temperature. Melting of the mixture began between 40 and 50°C. and at 50 to 54°C. the mixture was definitely molten. All doses of histamine are expressed in terms of the free base. As a routine, injections were given subcutaneously to guinea pigs and into the spinal or abdominal muscles of dogs. The tissues at the sites of injection were examined at various intervals after administration of the beeswax mixture.

TABLE 1

Comparison of the effects of histamine in saline solution and in beeswax mixture on guinea pigs

| ANIMAL | BODY WEIGHT | HISTAMINE INJECTED | | HISTAMINE REACTION | |
		Mgm. per 100 grams of body weight	In	Degree	Outcome
	grams				
1	604	0.5	Beeswax	Mild	Recovery
2	585	0.4	Beeswax	Mild	Recovery
3	605	0.3	Beeswax	Mild	Recovery
4	618	0.3	Beeswax	None	Recovery
5	540	0.3	Beeswax	Mild	Recovery
6	587	0.4	Saline	Severe	Fatal
7	542	0.3	Saline	Severe	Fatal
8*	538	0.4	Extract of beeswax	Severe	Fatal
9*	573	0.3	Extract of beeswax	Severe	Recovery

* Quantities of the mixture of histamine and beeswax containing 0.4 and 0.3 mgm. of histamine per 100 grams of body weight of guinea pigs 8 and 9, respectively, were extracted with hot saline solution to melt the beeswax and the resulting saline extract was injected.

RESULTS. *The guinea pig.* The mixture of histamine and beeswax was tested first on guinea pigs. Doses of histamine ranging from 0.3 to 0.5 mgm. per 100 grams of guinea pig were used. Such quantities of histamine, when dissolved in saline solution and injected subcutaneously, uniformly produced severe reactions which usually terminated fatally. When these doses were placed in the beeswax mixture and injected subcutaneously or intramuscularly, there was little or no reaction and none of the animals died (table 1). The results were so striking that it seemed possible that part of the histamine might have been destroyed by the beeswax. To test this, quantities of the beeswax mixture containing 0.4 and 0.3 mgm. of histamine per 100 grams of body weight of two guinea pigs were extracted with hot saline solution to melt the beeswax. Injection of the resulting saline extracts produced severe or fatal reactions in the guinea pigs (table 1, animals 8 and 9). Placing the histamine in the beeswax mixture had not destroyed it.

The dog. The results with the guinea pigs showed that some protection was obtained by placing histamine in the mixture of beeswax and mineral oil but the tests did not give any quantitative indication of prolonged action of histamine. For this purpose dogs that had Heidenhain pouches of the stomach were used and after the injection of a mixture containing histamine, the duration, volume and acidity of the secretion from the pouches were taken as measures of the action of histamine. Four dogs were studied in detail. In these the gastric juice secreted was collected every hour until the stimulating effects of the in-

TABLE 2

Gastric juice secreted from Heidenhain pouches in dogs in response to histamine in the beeswax mixture, histamine in saline solution and the beeswax mixture alone

DOG	EXPERI-MENT	HISTAMINE INJECTED				STIMULATION OF GASTRIC SECRETION				Histamine reaction
		Dose	Dose contained in		Number of sites dose divided between	Hours con-tinued	Total juice secreted			
			Volume	Material			Volume	Equiv-alent volume N/10 HCl	Grams HCl	
		mgm.	*cc.*				*cc.*	*cc.*		
1	1	15	0.75	Beeswax mixture	20	24	811	1147	4.2	Nil
	2	30	1.10	Beeswax mixture	20	29	861	1230	4.5	Mild
	3	33	0.94	Beeswax mixture	20	52	1312	1873	6.8	Nil
	4	60	0.82	Beeswax mixture	20	50	1570	2134	7.8	Severe
	5	15	1.00	Saline	10	7	103	143	0.5	Severe
	6	0	1.00	Beeswax mixture	20	0				Nil
2	1	30	0.80	Beeswax mixture	20	27	683	988	3.6	Nil
	2	30	1.20	Beeswax mixture	2	51	820	1199	4.4	Nil
	3	30	1.00	Saline	10	3	43	64	0.2	Severe
	4	0	1.00	Beeswax mixture	20	0				Nil
3	1	15	1.00	Beeswax mixture	20	29	1350	1969	7.2	Mild
	2	15	0.54	Beeswax mixture	20	29	875	1255	4.6	Nil
	3	15	1.00	Saline	10	3	98	132	0.5	Severe
4	1	35	1.00	Beeswax mixture	20	30	711	1062	3.9	Nil
	2	56	0.9	Beeswax mixture	20	46	1211	1627	5.9	Nil

jection had subsided. With two other animals hourly collections of juice were made at various intervals over the period of stimulation. In all, seventeen injections of histamine in the beeswax mixture were made into dogs that had Heidenhain pouches. The doses used ranged from 15 to 60 mgm. of histamine base. These amounts of histamine were contained in from 0.3 to 1.2 cc. of the mixture of histamine and beeswax. As a rule, when the mixture was injected, the volume containing the desired dose of histamine was divided among ten to twenty different intramuscular sites; about 0.05 cc. being injected at each point. This was done to keep the volume of foreign material at any one spot in the

muscle small and thus to keep the possibility of formation of abscess at a minimum. As the research progressed it was found that soon after injection the beeswax mixture became distributed along the long axis of the muscle fibers and bundles and that quantities of 0.1 to 0.5 cc. of the mixture in a single injection site were well tolerated.

When doses of histamine ranging from 15 to 60 mgm. were administered in watery solution to dogs, severe if not fatal reactions invariably ensued. When these doses were given in the beeswax mixture, only one severe and two mild reactions occurred. None of the reactions was fatal and all had subsided an hour after the injection. The severe reaction occurred when 60 mgm. of histamine was given in 0.8 cc. of beeswax mixture (table 2, dog 1, expt. 4). In this reaction there were repeated emesis, defecation, urination, some labored breathing and a rapid, rather feeble pulse with prostration for about one hour.
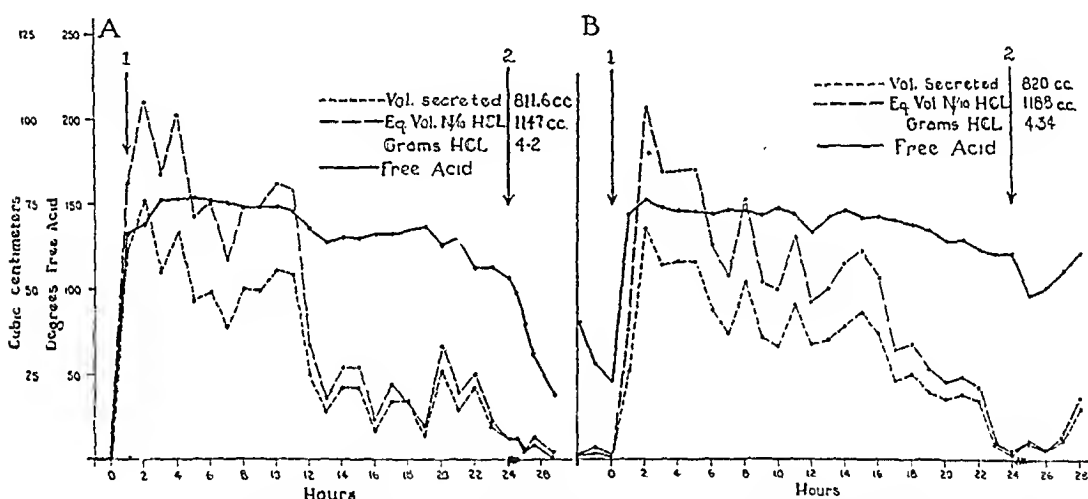
Fig. 1. Gastric secretory response from Heidenhain pouch of dog 1 when given 15 mgm. histamine in the beeswax mixture (A), and 30 mgm. histamine in the beeswax mixture (B). In both instances the material injected was divided among twenty different intramuscular sites. The time of injection is indicated by arrow *1*. The total volume and hydrochloric acid content of the juice secreted during the first twenty-four hours are shown at arrow *2*.

There was an increase of respiration in both mild reactions, with urination, defecation and vomiting in one but prostration of the animal did not occur in either. In all other instances the only obvious sign of action of histamine was a flow of gastric juice from the pouch.

As a rule the secretion of gastric juice commenced during the first fifteen minutes, reached its maximum during the first ten hours and continued for twenty-four or more hours after the injection of the mixture of histamine and beeswax (for example, figs. 1 and 2). In the case of the four animals whose responses were followed in detail, volumes of gastric juice ranging from 683 to 1570 cc., equivalent to 1 to 2 liters of tenth-normal hydrochloric acid, were secreted over periods of twenty-four to fifty-two hours when histamine was given in the beeswax mixture (table 2). The concentration of hydrochloric acid in the gastric

juice secreted in response to the mixture of histamine and beeswax varied over a narrow range. As a rule, the concentration of free hydrochloric acid rose to approximately 0.55 per cent (150 degrees free hydrochloric acid in the customary clinical units) and remained close to this value during the first twenty-four hours after injection when secretion was most active (figs. 1 and 2). Then, as the rate of secretion declined, the concentration of hydrochloric acid also declined. The animals whose responses to the mixture of histamine and beeswax were followed during the entire period of stimulation secreted a total of 3.6 to 7.8 grams of hydrochloric acid (table 2). The concentration of hydrochloric acid in the total volume of juice secreted ranged from 0.49 to 0.54 per cent (134 to 149 degrees free hydrochloric acid).
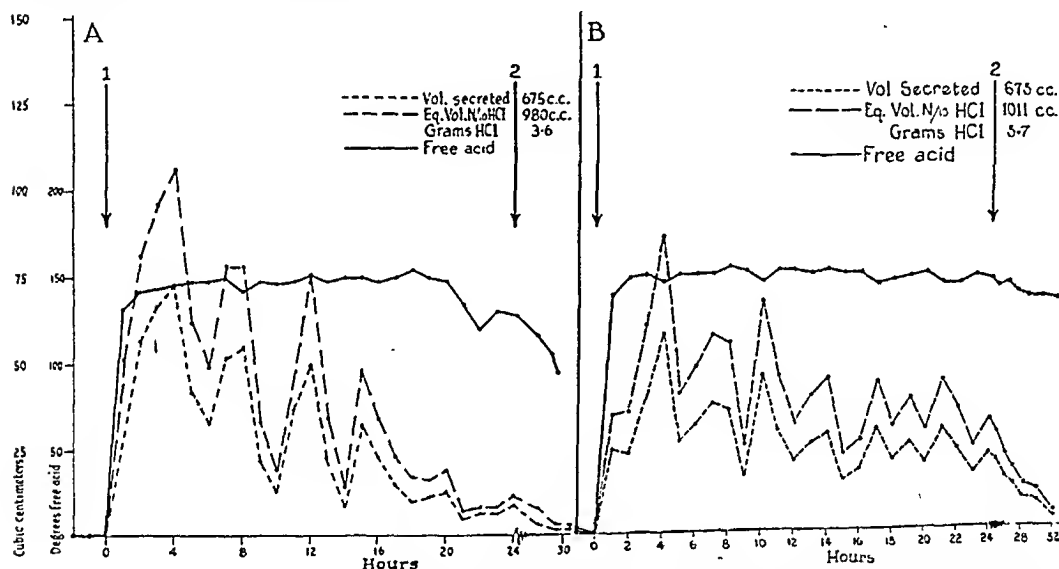
Fig. 2. Gastric secretory response from Heidenhain pouch of dog 2. At arrow *1*, 30 mgm. histamine in the beeswax mixture was given divided among twenty different intramuscular injection sites (A), and divided between only two intramuscular injection sites (B). The total volume and hydrochloric acid content of the juice secreted during the first twenty-four hours are shown at arrow *2*.

The volume of gastric juice secreted in response to injection of the mixture of histamine and beeswax involved the loss of considerable quantities of water, chloride and hydrogen ion. These losses were combated by adding salt to the drinking water, giving saline solution by vein and under the skin and by the return of gastric juice by tube to the stomach. A standard routine for replacement of the losses was not followed, however, and this fact may account for part of the variability in the results (table 2). The results from two experiments were discarded from the series because gross bleeding from the pouch occurred during the period of active secretion. During another experiment the animal passed a series of loose tarry stools indicative of bleeding in the upper reaches of the gastro-intestinal tract.

With one animal the secretion obtained from 30 mgm. of histamine in beeswax when divided among twenty sites of injection was compared with that obtained

when the same dose was given into only two sites (fig. 2). Judging from the secretory response the absorption of the histamine was at a more constant rate and extended over a somewhat longer period when the mixture was injected in only two places than when it was injected in twenty places.

The quantities of gastric juice secreted in response to injection of the mixture of histamine and beeswax were so large that the question arose, does the beeswax itself contribute to the gastric stimulation? To check this possibility a batch of the mixture of beeswax and mineral oil *without* histamine was made up and 1 cc. injected into each of two dogs. As usual, the material injected was divided between twenty different intramuscular sites and the juice secreted from the pouches was collected each hour for twenty-four hours. The total volume of juice secreted over the twenty-four hour period was 15 cc. in one animal and 52 cc. in the other (fig. 3). At no time during the twenty-four hour period after the
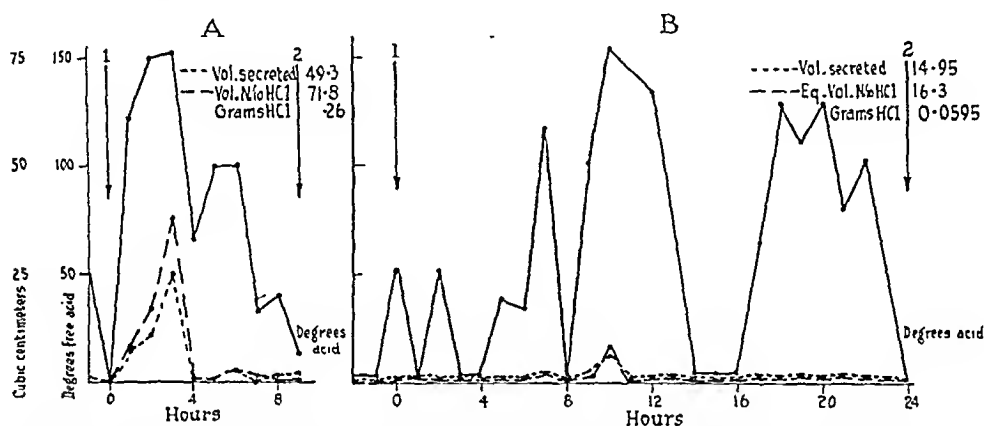
Fig. 3. Control observations on the gastric secretory response from Heidenhain pouch of dog 2. In A, at arrow *1*, 30 mgm. histamine in saline solution was injected intramuscularly. In B, 1 cc. beeswax mixture *without* histamine was injected intramuscularly at arrow *1*. The total volume and hydrochloric acid content of the juice secreted during the period of observation is indicated at arrow *2*.

injection of beeswax did the rate of gastric secretion exceed that often found in these animals under ordinary fasting conditions. It seems safe to conclude that the major part, if not all, of the stimulating action of the mixture of histamine and beeswax on gastric secretion is due to the presence of histamine in the mixture.

In order to gain a better measure of the degree of protection obtained by the use of the beeswax, doses of histamine similar to those used with the beeswax were given in saline solution to three of the dogs. Two of the animals received 15 mgm. and one 30 mgm. of histamine. The histamine was dissolved in 1 cc. saline solution and to simulate the injection of the beeswax mixture this volume was divided between ten intramuscular sites of injection. In all instances a severe reaction consisting of respiratory difficulty, repeated emesis, defecation and urination with marked prostration developed three to six minutes after the injection and lasted for thirty to forty minutes. During the first

thirty minutes, when the reaction was most severe, there was little or no gastric secretion. After recovery from the reaction secretion increased, reaching a maximum during the second or third hour and then sharply declining so that by the end of four or four and a half hours secretion had returned to the fasting level (fig. 3). The total volumes of juice secreted during this period were 103, 43 and 98 cc., respectively, compared with 811, 683 and 1350 cc. secreted when the same doses of histamine in the beeswax mixture were given to the same animals (table 2). Approximately eight to sixteen times as much juice was secreted when the histamine was placed in beeswax as when it was dissolved in saline solution.

COMMENT. Suspending histamine in mineral oil did not prolong its action. Apparently the tissue fluid reached the histamine particles in the oily suspension, dissolved them and carried the histamine away to produce its physiologic effects about as quickly as when the histamine was given dissolved in saline solution. A similar result was obtained when the particles of histamine were coated with paraffin and then suspended in mineral oil. The apparent cause of this was that at body temperature the paraffin dissolved in the mineral oil, leaving the histamine in suspension and thus affording little more protection than simple suspension in the oil. Histamine acid phosphate, which is very soluble in water, was used in these experiments and it would appear that if prolonged action were to be obtained by suspension in oil some less soluble form of histamine would have to be used.

It was while we were looking for a protective coating for the histamine particles which would not melt off at body temperature that beeswax was first used. Placing the histamine in the beeswax mixture certainly extended its period of action. The likely explanation for the phenomenon seems to be that displacement of the coating of beeswax mixture with admission of tissue fluid to the histamine particles takes place gradually through the mass of injected material and in this way a gradual liberation of histamine is obtained.

The total volume of gastric juice secreted by a pouch of the stomach in response to a dose of histamine in beeswax was a great deal larger than that produced by similar doses of histamine administered in saline solution. This feature of the results emphasizes the important physiologic difference between the sudden release of a large quantity of histamine and its prolonged continuous liberation. The acute effects of a rapid release or injection of histamine have been studied carefully but the usual methods of administration have not allowed a satisfactory investigation of the effects of continuous liberation of small quantities of histamine over prolonged periods. Use of the mixture of beeswax and histamine has afforded a means of studying the physiologic and pathologic results of chronic action of histamine (1, 2, 3).

                                   SUMMARY

In this study the amount, acidity and duration of secretion from gastric pouches of dogs following injection of histamine in a beeswax mixture were used as quantitative measures of the intensity and period of action of the histamine

contained in the mixture.   In response to 15 to 60 mgm. doses of histamine in
the mixture the pouches secreted gastric juice for twenty-four or more hours.
The juice secreted during this time varied between 683 and 1,570 cc. and its
hydrochloric acid concentration ranged from 0.49 to 0.54 per cent (134 to 149
degrees free hydrochloric acid in the customary clinical units).   A comparison
of the secretory response of the pouches to 15 and 30 mgm. doses of histamine
in beeswax with the response obtained to similar doses in saline solution showed
that in saline solution the response was completed in four and a half hours or
less in contrast to twenty-four or more hours when the beeswax mixture was
used and that the volume obtained when saline solution was used was only about
an eighth to a sixteenth of that secreted when the histamine was placed in the
beeswax mixture.   To determine whether the beeswax itself contributed to the
gastric stimulation, a mixture of beeswax and mineral oil containing no histamine
was injected.   It had little or no effect on gastric secretion.   It has been con-
cluded that the duration of the action of histamine may be prolonged by placing
it in a beeswax mixture.

<div align="center">REFERENCES</div>

(1) WALPOLE, S. H., R. L. VARCO, C. F. CODE AND O. H. WANGENSTEEN. Proc. Soc. Ex-
      per. Biol. and Med. 44: 619, 1940.
(2) VARCO, R. L., C. F. CODE, S. H. WALPOLE AND O. H. WANGENSTEEN. This Journal
      133: P475, 1941.
(3) HAY, L. H., R. L. VARCO, C. F. CODE AND O. H. WANGENSTEEN. Surg., Gynec. and
      Obst. (In press.)

THE SPONTANEOUS MOTILITY OF THE PYLORIC SPHINCTER AND ITS RELATION TO GASTRIC EVACUATION: THE "PYLORIC DIAGRAPH"[1]

J. P. QUIGLEY AND M. R. READ

From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio

The normal state of the pyloric sphincter and its influence on the propulsion of chyme has been variously described. According to the older view (summarized by Alvarez (1)) the sphincter was normally closed, either because it characteristically possessed a high degree of tonus or it was normally in a state of actual contraction. The sphincter thus functioned as the "keeper of the gate" and only permitted material to pass through either when it periodically relaxed or when sufficient pressure developed in an adjacent portion of the gut overcame the sphincter resistance and forced it to open.

Much of the recent literature, reviewed in part by Meschan and Quigley (2) or by Werle et al. (3), indicates the sphincter is open much of the time and does not constitute the major factor regulating gastric evacuation. These conclusions resulted from studies in which such foreign bodies as rubber balloons were present in the sphincter lumen. Since these objects might modify the motility and thus be responsible for the difference in the conclusions reached by the two groups of investigators, it became desirable to restudy the subject by a method which did not require the presence of a foreign body in the sphincter lumen.

EXPERIMENTAL. The following methods were employed. Using sterile technic, two lead shot were secured to the serosa crosswise of the pyloric sphincter so the shot would approach each other during sphincter contraction and be farther apart during sphincter relaxation. Movement of the shot was noted during experimental examinations by observing the alterations in their shadows cast on a fluoroscopic screen. This movement was registered by a device designed to record the distance across the sphincter, the "pyloric diagraph" or "shot chaser" (fig. 1). The portion of the apparatus resting on the fluoroscopic screen was moved about freely by the handles, A. The tips, B, were kept directly over the shadows of the lead shot. Changes in the distance between the tips B produced pressure changes in bellows C which were recorded by the optical manometer D. Respiratory movements moved the sphincter considerably but did not appreciably alter the distance between the shot; this type of movement, therefore, did not appear on the record. For most experimenters, the ability to use the pyloric diagraph effectively required several weeks of training, and the degree of concentration necessary during an experiment was such as to limit its use to consecutive 4 to 5 minute periods. While employing the pyloric diagraph,

234

the experimenter's head was in a chamber, light-proof except at the floor which was composed of the fluoroscopic screen.

A guide to the interpretation of the results obtained from the pyloric diagraph was obtained in eight experiments performed on unanesthetized dogs by making a pyloric diagraph record (fig. 2) simultaneously with the registration of the antral
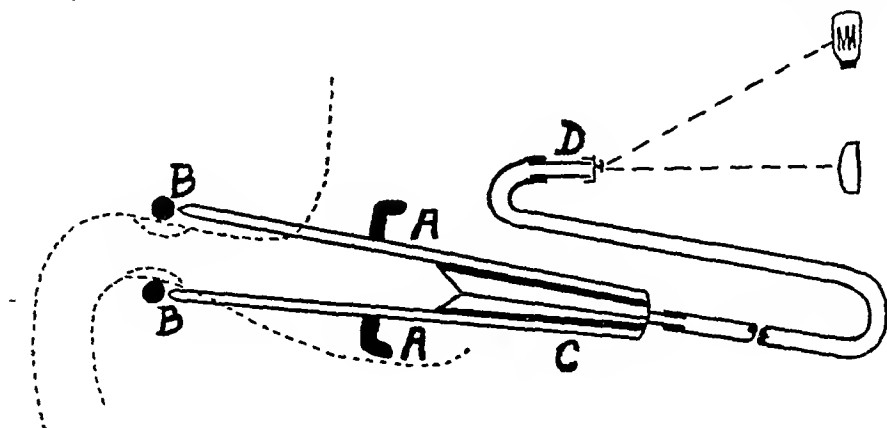

Fig. 1. The "Pyloric Diagraph." Handles *A* used to move the arms *B* so tips are kept directly over fluoroscopic shadows of lead shot secured to serosa crosswise of the pyloric sphincter. Alterations in distance between tips *B* produce pressure changes in bellows *C*. Pressure changes recorded from optical manometer *D*.
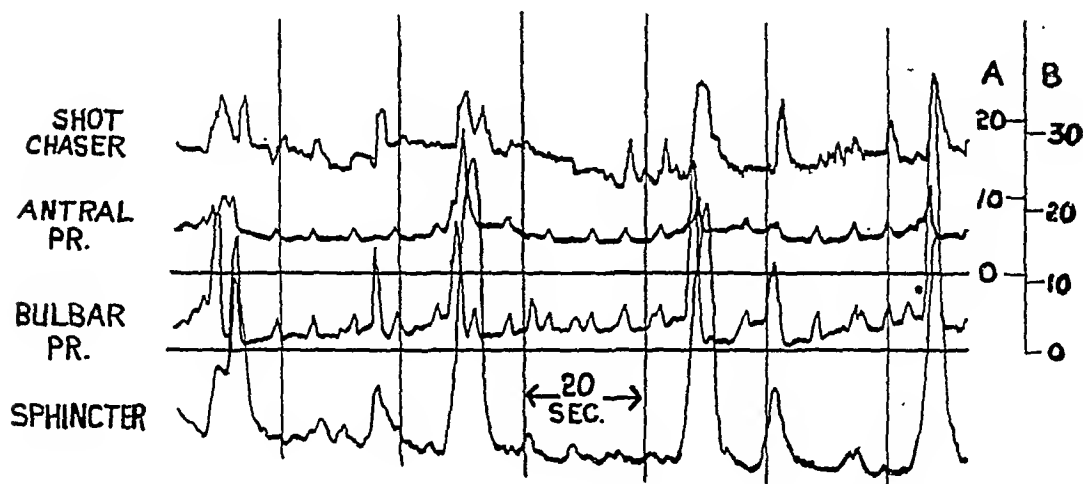
Fig. 2. Fasting animal. Upper record, pyloric sphincter motility as recorded by the shot chaser; lower record, sphincter activity recorded by the miniature balloon in the sphincter. Center records of antral and bulbar intralumen pressures. At right side, pressure scale of *A*, antral record, and *B*, of bulbar record.

and bulbar pressures by the method of Brody et al. (4), and a record from a miniature balloon (3 x 8 mm.) placed on the sphincter lumen and connected with an optical manometer. In addition, passage of a radio-opaque meal through the sphincter was studied fluoroscopically by a second observer who signalled the event by closing a key and thus caused a beam of light to strike the same photo-

graphic paper used to register the other phenomena. It was thus determined that records from the pyloric diagraph indicated rather accurately whether the sphincter was open or closed and when each phase of the movement began and terminated, but they gave only an approximate indication of the tonus changes and the magnitude of sphincter movement.

In addition to the types of experiments just described, twelve studies were made in which no recording device was placed in the sphincter lumen, but the antral and bulbar pressures were recorded from registering tips within the gut lumen, and the sphincter activity was followed with the pyloric diagraph (fig. 3). In an additional series of eight experiments, the pyloric diagraph alone was employed for registration and the gut lumen was free from recording devices. One-half of the latter two groups of studies was performed on fasting animals, but on the other ten occasions a $BaSO_4$ meal was administered and the passage

Fig. 3. Animal fed a $BaSO_4$ meal. Horizontal bars at top of record mark the intervals during which material was seen passing through the sphincter. Antral and bulbar intralumen pressures are indicated and at the right the scales indicate A, the antral pressure calibration, and B, the bulbar pressure. The sphincter record at the bottom of the record was obtained by the "shot chaser" method and adjacent to it is marked the onset and end of each evacuation cycle.

of material through the sphincter was recorded by the fluoroscopic method indicated above.

RESULTS AND INTERPRETATIONS. No difference was detected in the *fundamental type of motility* of the pyloric sphincter when recording equipment was present or absent from the lumen, nor in the fasting animals as compared with those evacuating gastric contents. The quiescent sphincter either in the fed or fasting animal was in the *relaxed state*. These results, obtained from direct observations, are contrary to the former teaching and they preclude the sphincter functioning under these conditions in a manner compatible with the usual implication of the term, "the keeper of the gate." The sphincter usually exhibited motility (especially in the fed animal) and this took the form of rhythmic waves which progressed in an orderly sequence over the antrum, sphincter and bulb. The fraction of each cycle during which the sphincter was relaxed varied inversely with the frequency of sphincter contraction, but in general the sphincter was relaxed for at least 60 per cent of the time.

Opening of the sphincter was not dependent to an important degree on the

force exerted by material expelled from a neighboring region. This claim is supported by the observation that the sphincter usually opened when nothing was being propelled in its immediate vicinity and also by the fact that while the sphincter was relaxing the antral and bulbar pressures were at or near their basal levels of 1.5–3 cm. of water. Contrary to the previous teaching, nothing passed through the sphincter in the period during which the sphincter was relaxing nor during the immediately succeeding interval.

Gastric evacuation normally began some time after the sphincter relaxation was complete (approximately in the latter half of the period during which the sphincter was open) and evacuation also continued during the period of sphincter contraction. During the first portion of the interval in which material passed through the sphincter, and while the sphincter balloon showed an increase in pressure, the pyloric diagraph record frequently but not invariably showed a further slight separation of the shot. This indicated that a moderate degree of passive distention of the relaxed sphincter sometimes accompanied gastric evacuation.

Reference to figures 2 and 3 shows that much of the evacuation of each cycle occurs during phase A, preceding the onset of sphincter contraction. As the sphincter contracts, it offers resistance to gastric evacuation, but does not prevent it. Approximately $\frac{1}{2}$ to $\frac{1}{3}$ of the material evacuated during each cycle passes through the sphincter while it is contracting (phase B), but it is evident that the resistance offered by the contracting sphincter reduces the amount of material evacuated and causes some to return to the body of the stomach. The sphincter, while contracting, increases the resistance to the escape of the antral contents and in conjunction with the antral peristaltic wave this results in an elevation of antral pressure (the antral pressure wave). Evacuation ceases at approximately the time the sphincter contraction is complete. The termination of evacuation is due chiefly to the fact that the distal antrum has emptied its contents. The closed sphincter could prevent further evacuation but usually this emptying process has terminated. Since the sphincter closed after bulbar filling and remained closed during bulbar contraction and the development of the bulbar pressure wave, it effectively retards regurgitation of bulbar contents.

SUMMARY

Studies of the pyloric sphincter motility made with the pyloric diagraph show that normally the quiescent sphincter is continuously in the relaxed state. The active sphincter normally exhibits cyclic activity and is relaxed during more than half of each cycle. Sphincter opening is a characteristic phase of the cyclic activity, not fundamentally the result of a passive stretching produced by material propelled from an adjacent portion of the gut lumen.

REFERENCES

(1) ALVAREZ, W. C. The mechanics of the digestive tract. New York, 1928.
(2) MESCHAN, I. AND J. P. QUIGLEY. This Journal 121: 350, 1938.
(3) WERLE, J. M., D. A. BRODY, E. W. LIGON, JR., M. R. READ AND J. P. QUIGLEY. This Journal 131: 606, 1941.
(4) BRODY, D. A., J. M. WERLE, I. MESCHAN AND J. P. QUIGLEY. This Journal 130: 791, 1940.

NORMAL HUMAN ARTERIAL OXYGEN TENSION[1]

STUART C. CULLEN AND EVELYN V. COOK

From the Division of Anesthesiology, Department of Surgery, University of Iowa College of Medicine, Iowa City, Iowa

In the investigation of alterations in arterial oxygen tension during artificially induced hyperpyrexia (1), it became evident that the control tensions calculated from the hemoglobin dissociation curve were somewhat lower than those reported in the literature. It has been stated (2) that 90 mm. Hg may be regarded as the average normal oxygen tension in arterial blood at sea level. This opinion was probably first expressed by Peters and Van Slyke (3) who summarized the data then available and stated that at sea level the arterial oxygen tension varied from 75 to 95 mm. Hg. They were uncertain, however, whether the difference between alveolar and arterial tension really covered the range as great as 5 to 25 mm. Hg in normal resting men or whether the reported variations were in part due to experimental error in some of the methods of measurement. One group of investigators reported normal arterial oxygen tensions between 95 and 100 mm. Hg (4). Other investigators reported values of 80 mm. Hg or less (5, 6).

Arterial oxygen tensions may be determined either directly by equilibration of blood samples with known gas samples or by calculation from the dissociation curve for hemoglobin with per cent oxygen saturation and carbon dioxide tension as the known factors. The more recent investigators have devoted most of their efforts to the study of the dissociation of hemoglobin with the result that the original dissociation curve (5) has been firmly established as being fundamentally correct and typical of normal human blood (7). This fact seems to justify the use of arterial oxygen saturations as a means of calculating oxygen tension. Only when values significantly different from the accepted ones are obtained would it seem necessary to check the calculated tensions by means of direct measurement.

In the previous investigation (1) in which thirty-two determinations of arterial oxygen saturation and tension were made on nineteen patients, the average saturation was 93.6 per cent and the average calculated tension 68 mm. Hg. Since these figures were significantly lower than the accepted values, it was thought advisable to study by direct measurement the arterial oxygen tension of normal human blood.

METHODS. The samples of arterial blood were taken from healthy interns, nurses, hospital personnel and ambulatory patients. All individuals, as far as could be determined, had normal respiratory and circulatory mechanisms, except case no. 21, who had hypertensive cardiovascular disease which was well

compensated. The samples were drawn from the brachial artery in the anticubital space or the radial artery at the wrist by the method of Adriani (8). This method insures anaerobic collection and preservation without the use of oil which has been suggested as a possible source of error (9, 10). The samples were kept in the syringes on ice until removed for analysis. Oxygen contents, oxygen capacities and carbon dioxide contents of 1 cc. samples were determined

TABLE 1

	PLASMA CO_2	O_2 CAP	PER CENT O_2 SATURATION	PER CENT O_2 SAT. AFTER EQUILIB.	PLASMA CO_2 VOL. PER CENT AFTER EQUILIB.	MM O_2 IN GAS	MM CO_2 IN GAS	ESTIMATED MM O_2 TENSION IN BLOOD
	vol. %	vol. %						
1. P. S.	59.0	21.45	94.0	93.6	57.3	70.0	36.8	73
2. H. P.	59.5	21.40	93.5	90.8	58.7	62.7	40.0	67
3. S. C.	57.0	21.50	94.5	92.0	52.7	69.0	37.8	75
*4. E. C.	60.0	17.40	94.0	93.0	59.5	65.5	38.4	68
5. H. W.	57.0	22.80	94.8	94.5	56.0	68.0	40.0	69
*6. A. D.	55.5	16.60	94.5	92.7	55.3	67.2	37.8	70.0
7. M. S.	56.5	21.65	96.5	95.5	56.5	71.0	39.0	72.0
8. J. S.	56.0	20.40	95.1	95.0	56.0	71.5	38.6	71.5
*9. H. E.	52.2	19.64	94.5	92.4	52.0	67.0	38.8	70
10. F. O.	59.0	19.10	94.8	95.6	57.6	70.0	40.6	70
11. J. S.	60.0	21.00	94.0	95.5	60.0	69.0	41.0	68
12. A. Y.	63.0	20.24	94.0	93.4	63.0	70.0	42.8	71.0
13. H. K.	51.0	20.20	94.0	93.2	53.0	70.0	40.0	71.0
14. B. F.	56.5	18.70	96.2	97.3	57.7	75.5	40.7	73.5
*15. M. B.	56.5	18.45	95.8	94.5	57.0	74.5	38.8	74.5
*16. C. E.	55.0	18.78	96.0	96.0	54.5	74.0	38.8	74
17. B. C.	59.8	20.12	93.8	94.0	59.7	72.8	41.8	72.5
18. L. L.	60.7	18.15	95.7	96.2	59.9	75.7	41.2	75.5
19. H. V.	63.0	20.82	94.0	95.5	52.0	72.0	43.6	72
*20. M. H.	62.0	19.22	94.1	93.6	61.7	71.6	44.0	73
21. J. H.	66.3	24.20	89.5	90.5	65.3	68.2	44.5	68
22. A. K.	53.2	19.47	95.4	94.2	53.0	74.0	39.0	75
23. D. D.	55.2	23.20	95.0	94.7	56.2	76.5	39.8	76
24. A. M.	59.3	19.35	96.4	96.1	59.5	79.6	41.5	79
25. C. T.	55.3	19.73	91.8	97.0	56.7	80.1	41.5	74
Average			94.5					72.1

* Denotes females.

on the manometric apparatus according to the method of Van Slyke and Neill (9). After correcting for dissolved oxygen, the percentage saturation of hemoglobin was then used to calculate the oxygen tension. It was assumed that all individuals had a 40 mm. (±) tension of carbon dioxide.

A gas sample of oxygen, carbon dioxide and nitrogen was then made up to correspond with the calculated tensions of those gases in the blood sample. This gas sample was made up directly in a tonometer with a capacity of 165 cc. Four cubic centimeters of blood were then admitted to the tonometer and before rotating, the tonometer containing blood and gas samples was submerged in a

37.5°C. water bath.   Gas was allowed to escape so that the gas tension at 37.5°C. would equal atmospheric pressure.   After sealing the gas outlet stopcock with mercury, the tonometer was then rotated slowly in the water bath for 25 or 30 minutes.   A 1 cc. sample of the equilibrated blood was then withdrawn and analyzed.   Following this, 25 cc. of the equilibrated gas sample was withdrawn and also analyzed in the manometric apparatus.   Gas tensions were calculated as the per cent found times the barometric pressure minus the vapor pressure, using 750 mm. Hg as barometric pressure and 49 mm. Hg as vapor pressure of the blood.

RESULTS.   In the data obtained from this procedure (table 1) it will be noted that except in a few instances when the gas sample tension agreed perfectly with the blood tension, there was a small change in the oxygen saturation of blood sample.   Since the shift in carbon dioxide tension is closely related to the percentage oxygen saturation at a given oxygen tension, the original blood oxygen tension was estimated in those latter samples on the basis of the slight change in per cent saturation and carbon dioxide content of the equilibrated blood.

The average arterial oxygen saturation was 94.5 per cent and the average oxygen tension 72 mm. Hg.   These figures agree very closely with the corresponding point on the dissociation curve for human hemoglobin at a 40 mm. Hg tension of carbon dioxide.   In only one sample was there an 80 mm. Hg oxygen tension and that hemoglobin was 96.4 per cent saturated and the carbon dioxide content was relatively high.   In another instance, an 80 mm. Hg oxygen tension was made up in a gas sample with a 40 mm. Hg carbon dioxide tension. This raised the blood sample from 91.8 per cent saturation to 97 per cent saturation.

DISCUSSION.   Although the average value of 94.5 per cent saturation is not very much lower than those saturations reported (7, 11) with the exception of one group (12), the average tension of 72.5 mm. Hg obtained by direct measurement is appreciably lower than the reported tensions (2, 3, 4, 5, 6).

It will be recalled that the original investigation gave an average saturation of 93.6 per cent with a tension of 68 mm. Hg (1).

All of the patients in that investigation were receiving treatment and many were at bed rest.   As a matter of interest, tension determinations were made on a few of the normal humans seven to ten days after an uncomplicated appendectomy.   Their saturations dropped 2 to 4 per cent and the tensions dropped 5 to 10 mm. Hg and approximated the values obtained in the patients of the previous study.

<div align="center">SUMMARY</div>

The data on 25 normal human arterial blood samples indicate that the average oxygen saturation is 94.5 per cent and the average oxygen tension is 72 mm. Hg.

<div align="center">REFERENCES</div>

(1) CULLEN, S. C., E. F. WEIR AND E. COOK.   Anesthesiology 3: 123, 1942.
(2) BARD, P.   McLeod's Physiology in modern medicine, p. 622, 1941.

(3) PETERS, J. P., AND D. D. VAN SLYKE. Quantitative clinical chemistry 1: 532, 1931.

(4) BARCROFT, J., A. BINGER, A. V. BOCK, J. H. DOGGART, H. S. FORBES, G. HARROP, J. C. MEAKINS, AND A. C. REDFIELD. Trans. Roy. Soc. (B) 211: 351, 1923.

(5) BOCK, A. V., H. FIELD, JR. AND G. S. ADAIR. J. Biol. Chem. 59: 353, 1924.

(6) BOCK, A. V., D. B. DILL, H. T. EDWARDS, L. J. HENDERSON AND J. H. TALBOTT. J. Physiol. 68: 277, 1930.

(7) DILL, D. B., H. T. EDWARDS AND W. V. CONSOLAZIO. J. Biol. Chem. 118: 635, 1937.

(8) ADRIANI, J. J. Lab. and Clin. Med. 23: 1094, 1938.

(9) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. 2, 1932.

(10) LOONEY, J. M. AND H. M. CHILDS. J. Biol. Chem. 104: 53, 1933.

(11) KEYS, A. AND A. SNELL. J. Clin. Investigation 17: 59, 1938.

(12) LOONEY, J. M. AND E. M. JELLINEK. This Journal 118: 225, 1936.

THE RATE OF GLUCOSE ABSORPTION FROM THE INTESTINE OF DIABETIC RATS

FRANCES PAULS AND DOUGLAS R. DRURY

From the Scripps Metabolic Clinic, La Jolla, and the Department of Physiology, University of Southern California Medical School, Los Angeles

The rate of absorption of glucose from the intestine of the intact rat has generally been studied under conditions (1, 2) which resulted in the influx into the stomach of a large volume of concentrated glucose solution during a few seconds. Under such special conditions of forced feeding the absorption coefficient (mgm. glucose absorbed per sq. dm. body surface per hour) is generally around 100 (1–3) and could do little more than cover the basal energy requirement of the rat if glucose were absorbed at the maximum rate throughout the 24 hours. Rats may be maintained on a diet consisting almost exclusively of glucose and since this animal normally confines its eating to the night time it is obvious that the glucose absorption rate under normal conditions must be considerably higher than that found with large size doses of glucose given by forced feeding. Under more normal conditions of voluntary feeding in which the taking of large amounts of glucose was induced by the administration of protamine zinc insulin, alternate fasting and feeding periods and exposure to a very cold environment (3) glucose absorption coefficients 2 to 5 times as large as those obtained by forced feeding were found. The enormous food intake of diabetic rats presented another method of observing the rate of glucose absorption from the intestine under the conditions of voluntary feeding by simply offering a diet composed largely of glucose.

EXPERIMENTAL. Young male rats weighing between 100 and 150 grams were depancreatized by Greeley's adaptation (4) of the method of Shapiro and Pincus (5). They were allowed to remain on the stock diet of dog pellets until well recovered from the operation and then placed on a high carbohydrate diet (6). Under such circumstances these rats lose from 3–10 grams of glucose per square decimeter per day in the urine and consume correspondingly large amounts of food. Although no weight is gained on the high carbohydrate diet they are able to maintain their weight. The urine volume and hence the water intake varies with the amount of sugar lost in the urine and may go as high as 100 cc. per square decimeter of body surface per day.

Data for a typical rat (no. 1) is presented for a 10 day period in table 1. Typical results for 14 other animals are given for single days. The days selected for depicting here were chosen from periods when the day to day food intake was reasonably constant (e.g., no. 1, days 2–5 or 8–10). The absorption coefficients are calculated from the total glucose intake in the diet and it is assumed that absorption went on evenly throughout the 24 hour periods. The absorption

coefficients are very high in comparison with those which are obtained with forced feeding (1, 2). If, as is most likely, glucose absorption was proceeding during only a part of the 24 hours this coefficient would be even higher.

The amount of glucose lost in the urine has been recorded in order to indicate the severity of the diabetes.

TABLE 1

Rate of glucose absorption in diabetic rats when glucose is administered ad libitum as the bulk of the diet

RAT NUMBER	DAY	BODY WEIGHT	BODY SURFACE	URINE PER SQ. DM. BODY SURFACE PER DAY		GLUCOSE ABSORPTION COEFFICIENT*
				Volume	Glucose	
		grams	sq. dm.	cc.	grams	mgm.
1	1	168	3.4	49	4.7	294
	2			38	4.1	270
	3			37	3.5	284
	4			44	4.4	270
	5			47	4.7	294
	6			38	3.8	345
	7			44	3.8	294
	8			30	2.7	307
	9			41	2.7	307
	10			32	2.7	307
2		205	4.0	61	6.2	312
3		157	3.3	69	6.9	315
4		181	3.6	64	5.3 -	326
5		155	3.3	61	5.2	327
6		170	3.5	37	3.7	333
7		146	3.1	41	4.5	335
8		142	3.1	68	6.8	336
9		150	3.2	51	6.3	338
10		175	3.6	54	5.3	340
11		120	2.8	50	5.0	342
12		168	3.4	65	4.7	343
13		192	3.8	66	5.5	350
14		120	2.8	55	5.0	358
15		105	2.5	90	8.0	470

* The absorption coefficient is the milligrams of glucose absorbed per square decimeter of body surface (9) per hour calculated in this case from the glucose content of the total food intake over the 24 hour period.

DISCUSSION. It is improbable that the fact that these rats were diabetic had any influence upon the absorption rate of glucose from the intestinal tract. Their blood sugar concentrations are high (300–600 mgm. per cent) and in other ways they deviate from normal but large doses of insulin have no effect upon glucose absorption during the first day of administration when they are insulin resistant. Subsequent doses of insulin control the diabetes and reduce the

appetite, hence there is less to be absorbed. This reduction in glucose absorbed is simply incidental to the reduction in appetite and not a result of any effect of the insulin as such on the absorption rate for very high glucose absorption coefficients may result from insulin administration to normal animals because of the hyperalimentation (3) which is apparently due to a low blood sugar level (7). It is not obvious why the forced feeding of glucose leads to such low absorption coefficients (1–3) in comparison with the high absorption rates found when the glucose is taken voluntarily after fasting, encouraged by a cold environment, a result of protamine zinc insulin (3) or by these diabetic rats.

One might argue that high absorption coefficients have been demonstrated only under conditions when there is a need on the part of the organism for a large amount of glucose and hence that the high coefficients are a result of the demands of the organism for calories rather than due to voluntary ingestion of the glucose. The experiments of Althausen and Stockholm (8) point in this direction for in hyperthyroidism produced in rats by thyroid feeding they found quite high glucose absorption coefficients when the glucose was given by forced feeding. However, we have observed much higher coefficients in thyroid treated rats taking glucose voluntarily. It seems probable that the absorption mechanism of the intestinal tract operates most efficiently under conditions governed by many functions such as gastric motility, secretions of the various parts of the tract, etc., and the disturbance of these, which is usually produced by forced feeding leads to gross inefficiency and low absorption coefficients.

Disregarding water arising in metabolism or the loss of water by routes other than the urine, the urine volumes indicate that enough water is ingested so that the concentration of glucose in the ingesta is on the average kept below 14 per cent. We have administered glucose by forced feeding to normal rats in a concentration of 12 per cent but obtained absorption coefficients little higher than when strong solutions were given. A factor which must not be overlooked is that the fluid as well as some of the other constituents of the saliva, gastric juice, etc., continually circulate and may lead to sugar which is ingested voluntarily reaching the absorbing surface in a very diluted form as well as more or less continually.

SUMMARY

Diabetic rats store sugar with difficulty and on a high carbohydrate diet consume large amounts of sugar because of the loss in the urine. Even assuming that the intestinal absorption of glucose is proceeding at the same rate throughout the day and night the absorption coefficients are extremely high.

The rate of glucose absorption in diabetic rats is compared with the high absorption coefficients obtained under other conditions of voluntary glucose feeding and contrasted with the low coefficients which have been reported under conditions in which glucose has been given by forced feeding. Some of the possible reasons for the low glucose absorption rates found under the latter abnormal conditions, and which have long been considered the true picture of this intestinal function, are discussed.

REFERENCES

(1) CORI, C. F. J. Biol. Chem. **66**: 691, 1925.
(2) MacKay, E. M. and H. C. Bergman. J. Biol. Chem. **101**: 453, 1933.
(3) MacKay, E. M. and W. G. Clark. This Journal **135**: 187, 1940.
(4) Greeley, P. O. Personal instruction.
(5) Shapiro, R. and G. Pincus. Proc. Soc. Exper. Biol. and Med. **34**: 416, 1936.
(6) Drury, D. R. This Journal **131**: 536, 1940–41.
(7) MacKay, E. M., J. W. Callaway and R. H. Barnes. J. Nutrition **20**: 59, 1940.
(8) Althausen, T. L. and M. Stockholm. This Journal **123**: 577, 1938.
(9) Carman, A. A. and H. H. Mitchell. This Journal **76**: 380, 1926.
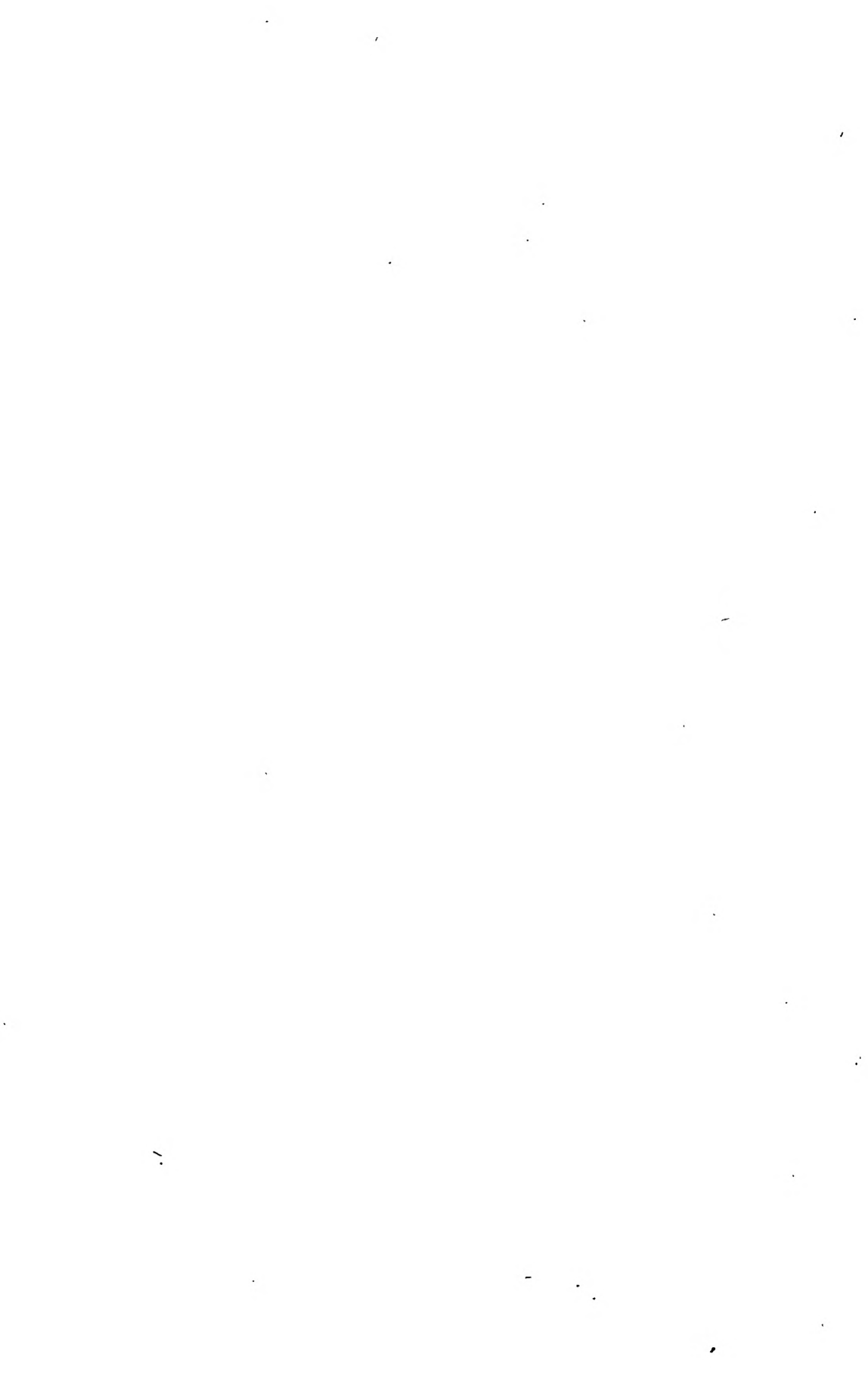
THE INNERVATION OF THE MUSCULI INTEROSSEI AND THE TOE SPREADING REFLEX

RUDOLF ALTSCHUL AND K. P. TURNER

From the Department of Anatomy, School of Medicine, University of Saskatchewan, Saskatoon, Canada

If a rabbit or a guinea pig is raised from the ground and especially if it is kept in a vertical position with the head upright, the toes of the hind legs spread widely apart in extreme abduction. This is the "toe spreading reflex". In young animals the toes of the fore legs are similarly affected, but since the spreading of the toes of the hind legs is the more constant phenomenon we have confined our attention to it.

The spreading reflex of the toes is stronger in rabbits and guinea pigs than in cats and therefore in these rodents it is more easily studied and its absence more readily observed. However, cats lose not only the faculty of abduction but also the ability to extend the claws. The abduction of the toes is brought about by the action of the dorsal interossei muscles which in man are supplied by the nervus tibialis (posterior tibial nerve).[1] In the opinion of R. Magnus the spreading reflex of the toes is of labyrinthine origin. Magnus states:

> The guinea pig is taken from behind under the shoulders, thus hanging freely with the head upright. The head is then in a normal position. The toes of both hind limbs are put together by delicate stroking. If now the animal is moved gently downwards the toes suddenly abduct. The reaction is not present in all animals, yet can be shown in most of them. Ordinarily a very little movement is sufficient to elicit this reaction, which was already described by Graham Brown.
>
> The spreading of the toes starts at the beginning of the movement or only after cessation of it, depending on the sensibility of the animal.
>
> After extirpation of both labyrinths the spreading of the toes is absent. . . .
>
> The described spreading of the toes occurs also when the head is fixed, thus excluding movements of the neck.

In the course of an investigation on muscle degeneration in which rabbits and cats were used, the sciatic or the common peroneal nerve was excised or evulsed and the spreading reflex was abolished (fig. 1). This reaction became a valuable indication of the condition of the nerve. Its absence provided visible evidence of interruption of the nervous pathway and its re-appearance indicated the regeneration of the nerve and the re-establishment of the nervous circuit.

The observations so made provided evidence as to the innervation of the dorsal interossei muscles and the mechanism of the spreading reflex of the toes which are at variance with the commonly accepted views and are therefore recorded.

As already mentioned the reflex arc was interrupted and the reflex disappeared

[1] The BNA has been used throughout, but where references have been made to human anatomy, the terms of the Birmingham Revision, B. R., have been added in brackets.

after cutting the common peroneal nerve. This section, therefore, interrupted either the afferent or the efferent limb of the reflex arc. To decide this question we carried out the following tests:

1. In a rabbit the nervus tibialis was cut to ascertain if this interruption would affect the reflex. It was found that spreading of the toes occurred normally in this animal.

2. In other animals the nervus peronaeus communis was cut and the reflex was abolished. Stimulation of the peripheral stump by the faradic current produced spreading of the toes, but no abduction followed stimulation of the central stump.

3. Faradic stimulation of the tibial nerve, after isolation of the latter, did not produce spreading of the toes.

In man the m. interossei of the foot are innervated by the nervus tibialis (posterior tibial nerve). According to Gray some fibers of the nervus peronaeus

Fig. 1. Showing the ventral aspect of a rabbit in which the right common peroneal nerve has been cut with a loss of the spreading reflex on that side, and the presence of the reflex on the other side in which the nerve was intact. Note the shadow. (The reproduction shows the original photograph rotated through 90°.—Ed.)

profundus (anterior tibial nerve) may reach the first, second and some times the third dorsal interosseus muscle. But Cunningham assumes that these small branches are probably afferent.

It is apparent, therefore, that in rabbits and cats the innervation of these muscles is different from that found in man and they receive their motor supply through the common peroneal nerve by way of the nervus peronaeus profundus.

The afferent impulse and pathway seem connected in some way with the sense of deep sensibility (proprioreceptive). The loss of pressure, due to removal of body weight from the paws, is, we believe, the eliciting factor. As already stated, it has been assumed by Magnus that the reflex is of labyrinthine origin, this opinion being based on the observation that the reflex is elicited when the animal is placed in a vertical position and the body moved downwards, the assumption being that the labyrinths are thus stimulated.

We found, however, that the spreading of the toes as a reflex action may be elicited also if the animals are picked up, grasping them by two places on the

back, in such a manner that the spine remains in a horizontal position and the head in a natural relation to it. Thus, the reflex may appear even when the labyrinth is orientated differently from that set forth by Magnus.

In very young rabbits and kittens we found that the spreading of the toes is present in nearly all positions of the body, provided the paws of the animals are not in contact with a base. But if, under the above mentioned conditions and also with the trunk in vertical position, a supporting base was offered to the animal's paws, without changing the position of the body itself, the toes were adducted.

These facts seem to contradict Magnus' statement that the spreading of the toes is a labyrinthine reflex. The disappearance of the abduction of the toes after a bilateral labyrinthectomy does not prove that the reflex is of labyrinthine origin. Following bilateral extirpation of the labyrinth, the tone of the muscles of the limbs greatly diminishes (Magnus, 1924) and with this loss of muscular tone one of the conditions of the reflex is lacking. Further evidence advanced by Magnus, namely, that: "if the toes are put together by stroking and thereafter the animal moved up or down, the spreading reflex appears promptly and extensively" may be explained on the grounds that such movements increase the labyrinthine tone of the muscles thus creating better conditions for the establishment of the reflex. But these brisk up and down movements may act otherwise. They may stimulate directly the organs of deep sensibility in muscles and in the connecting joints and ligaments, thus increasing the muscle tone by reflex action.

In further support of this idea of tone we found that when provoking the vertebra-prominens-reflex in a guinea pig, while held in an upright position, the hind limbs were flexed and the spreading reflex of the toes disappeared, apparently without any change in the position of the head and thus without stimulation of the labyrinths.

SUMMARY

1. The dorsal interossei muscles, which perform the abduction of the toes of the hind limbs, readily observed in the toe spreading reflex, are innervated in cats and rabbits by fibers of the common peroneal nerve, whereas in man the interossei of the foot are commonly innervated by the tibial nerve (posterior tibial nerve).

2. We believe we have shown that the spreading of the toes is a reflex in which the afferent path of the arc is from proprioreceptors in the hind limb, rather than a reflex of labyrinthine origin as described by Magnus. The efferent path is over fibers in the common peroneal nerve. The spreading reflex may be influenced by changes in the muscle tone in the hind limbs, which may be due to a bilateral labyrinthectomy, but the reflex proper does not depend directly on the labyrinths.

REFERENCES

CUNNINGHAM. Text-book of anatomy. VIIth ed., Oxford University Press, London, 1937.

GRAY. Anatomy, descriptive and applied. XXVIth ed., Longmans, Green & Company, London, 1935.

MAGNUS, R. Körperstellung. Springer, Berlin, 1924.

THE INFLUENCE OF HEMORRHAGE ON SKELETAL MUSCLE TONE

W. F. KIELY, S. L. HAMILTON and E. GELLHORN[1]

*From the Departments of Physiology and Psychiatry, University of Illinois, College
of Medicine*

Henderson (1938) emphasizes the rôle of skeletal muscle tone for the venous return and thereby for the maintenance of the circulation. He attributes the shock after operation and anesthesia, to a large extent, to a diminution in skeletal muscle tone and consequent inadequate filling of the heart. If the muscle tone plays such a predominant rôle in the maintenance of the circulation it seemed of interest to investigate the effect of circulatory disturbances upon it. For this reason the influence of hemorrhage on the muscle tone was studied in decerebrate dogs.

METHOD. The experiments were performed on 9 decerebrate dogs which were initially anesthetized with ether. About one hour was permitted to elapse before the experiment was begun in order to eliminate the effects of ether.

The blood pressure was recorded from the carotid artery, the muscle tone was measured by means of Henderson's technique in the extensor muscles of a hindleg. One arm of a water manometer was connected to the muscle by means of an 18 gauge needle provided with additional side holes. The needle was filled with Ringer's solution whose level could be read in a glass capillary fitted to the needle; the other arm of the water manometer was connected with a column of Hg the height of which could be varied, thereby providing pressures tending to force Ringer's solution from the needle into the muscle. The pressure necessary to just perceptibly lower the level of the meniscus of Ringer's in the capillary was taken as a measure of muscle tone as described by Henderson and collaborators (1936). The dog was bled through the femoral artery of the contralateral leg and the citrated blood was re-infused through the vein of the same leg.

Ephedrine and adrenalin, whose effects on muscle tone were studied, also were injected into the femoral vein.

RESULTS. Table 1 gives a summary of the results and shows that even slight losses in blood lead consistently to an increase in muscle tone. This effect is reversed on re-infusion of blood. The table also shows (cf. nos. 6 and 7) that the experiment can be repeated in the same animal with nearly identical results. It is interesting to note that if the dog is bled to death a very low blood pressure is accompanied by an excessive increase in muscle tone.

If, after the withdrawal of a certain quantity of blood, repeated recordings of the muscle tone are taken, it is seen that with the gradual spontaneous restoration of the blood pressure the muscle tone decreases.

From these observations one might be inclined to conclude that muscle tone and blood pressure are inversely related and that the level of the blood pressure

is the determining factor for the tone of the striated muscles. However, further experiments showed that hemorrhage may lead to the same increase in muscle tone if the fall in blood pressure is prevented.

TABLE 1
Effect of hemorrhage on muscle tone in decerebrate dogs

NO.	BEFORE BLEEDING		AT THE END OF BLEEDING PERIOD		AFTER REINFUSION OF BLOOD		BLOOD WITHDRAWN IN PER CENT OF BODY WEIGHT	REMARKS
	T*	BP†	T	BP	T	BP		
1	48	126	130	96	50	116	0.7	
2	40	90	60	75	50	82	0.8	
3	32	118	50	70	32	126	0.6	Carotid sinuses denervated, vagi cut
4	80	120	120	94	70	126	0.5	
5	40	192	80	160	40	176	0.5	
6a	60	70	150	39	60	70	0.9	
6b	60	74	175	34	60	70	0.9	
6c	75	48	120	28				Dog bled to death
			200	24				
7a	70	158	100	108	65	176	2.6	
7b	70	176	110	124	75	172	2.6	
7c	100	100	150	70				Dog bled to death
			200	48				

* T = Muscle tone in millimeters of H_2O.
† BP = Blood pressure in millimeters Hg.
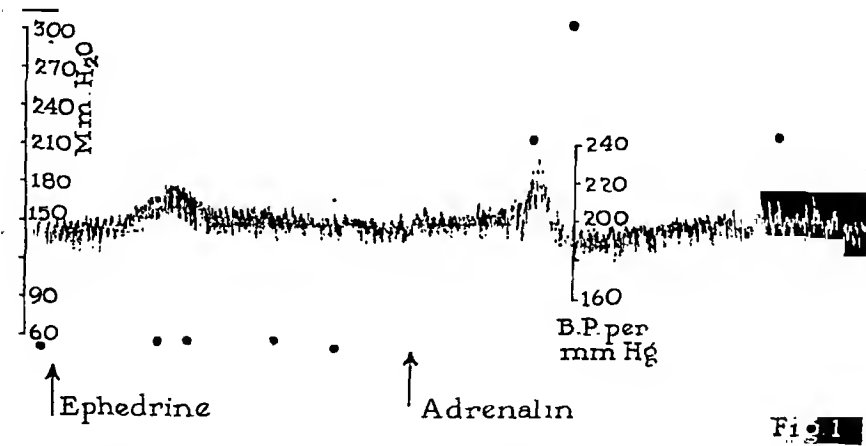
Fig. 1. Effect of ephedrine (0.9 mgm./kgm.) and of adrenalin (0.03 γ/kgm.) on blood pressure (mm. Hg) and muscle tonus (mm. H_2O) of a decerebrate dog. The muscle tone is indicated by white dots.

In these experiments ephedrine was used in order to eliminate or minimize the fall in blood pressure. As figure 1 shows, ephedrine does not alter the muscle tone although it causes a rise in blood pressure. It was found that even 1.5 and 2.2 mgm./kgm. of ephedrine was without effect on the muscle tone although

0.5 mgm./kgm. was adequate to prevent the fall in blood pressure during moderate hemorrhage. Contrariwise, adrenalin increases the muscle tone. The increase in muscle tone induced by adrenalin is not restricted to the vasopressor period but remains high for some time after the blood pressure has returned to its original level.

On the basis of these studies experiments were performed in which the influence of hemorrhage on muscle tone was studied with and without simultaneous infusion of ephedrine. The experiments reproduced in figures 2 and 3 were conducted on the same animal. It is seen that the removal of 200 cc. (1 per cent of body weight) causes the blood pressure to fall from 140 to 102 mm. Hg and the muscle tone to rise from 50 to 120 mm. H_2O. The same hemorrhage induced while ephedrine is being infused causes a fall in blood pressure from 144 to 130 mm. Hg and a rise in muscle tone from 60 to 129 mm. H_2O. Apparently the greatly diminished blood pressure reaction fails to influence the reactivity of the muscle tone. Moreover, the same experiment shows that the removal of 100 cc. of
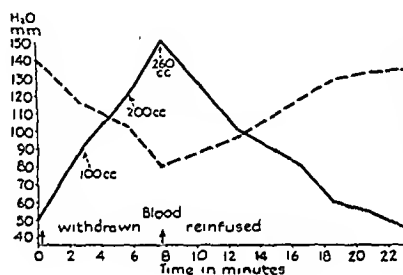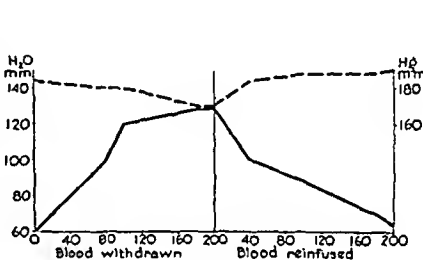
Fig. 2.   Fig. 3

Fig. 2. Effect of hemorrhage on blood pressure (- - - -) and muscle tone (——) of a decerebrate dog (20 kgm.).

Fig. 3. Same dog as in figure 2. Bleeding occurs while 0.5 mgm./kgm. ephedrine is being injected intravenously.

blood (0.5 per cent of body weight) causes a considerable rise in tone although the blood pressure remains unchanged.

These observations suggest that carotid sinus pressor reflexes are not primarily involved in the change of the muscle tone resulting from hemorrhage. Experiments have indeed shown that denervation of the carotid sinuses and bilateral vagotomy do not abolish the rise in muscle tone during bleeding (table 1). Furthermore, it was found in some instances that in dogs with bilateral denervation of the sino-aortic area bleeding led not only to a fall in blood pressure and rise in muscle tone but also to a grossly observable rigidity (occasionally with opisthotonus). In this case the fall in blood pressure was followed by a considerable temporary rise and during this phase the muscle tone rose greatly.

Stimulation of the peripheral end of the vagus did not alter muscle tone although the blood pressure was greatly reduced.

Discussion. It is well known (Koch, Bruner and Mertens, Gellhorn and Pollack) that denervation of the carotid sinus areas greatly diminishes the resistance to hemorrhage as shown by the more rapid fall of the blood pressure in

"denervated" animals. Sympathectomized animals are likewise more sensitive to hemorrhage than control animals (Schlossberg and Sawyer). Obviously the carotid sinus reflexes elicited by the fall in blood pressure during bleeding greatly contribute to the restoration of the blood pressure and thereby to the adequate filling of the heart. However, the restoration of the circulation depends not only on these autonomic reflexes but seems to involve the somatic nervous system as well since the tone of the striated muscle which depends on spinal centers is increased in hemorrhage.

In view of the evidence presented by Henderson for the rôle of the muscle tone for the venous return and thereby for the maintenance of cardiac output and blood pressure it must be assumed that the increased muscle tone in hemorrhage represents an important reaction of the somatic nervous system which contributes to the circulatory adjustment. Since the muscle tone is increased by hemorrhage even when the blood pressure is kept constant and after elimination of the pressor receptors of the sino-aortic area vascular reflexes on the tonus regulating spinal centers play only a minor rôle, if any.

Our investigations show that adrenalin (cf. Beiglbock) and ephedrine act differently on skeletal muscle tone although both raise the blood pressure through peripheral vasoconstriction. Obviously the change in muscle tone is not the result of the raised blood pressure but is due to the action of these drugs on the striated muscles or the spinal centers. This interpretation is supported by the fact that the muscle tone is still greatly increased following the injection of adrenalin when the blood pressure has returned to its original level. In view of the fact that adrenalin is secreted under conditions of impaired oxygenation of the tissues (hemorrhage, shock, anoxia, narcosis) its action on the striated muscles and indirectly on the venous return seems to play an important part in the restoration of the internal environment.

The mechanism by which the skeletal tone is altered is very sensitive to narcosis. A series of experiments was performed by Cortell and Gellhorn on cats anesthetized with chloralose (unpublished observations). Moderate bleeding did not change the tone of striated muscles under these conditions. The effect of inhalation of carbon dioxide on muscle tone was slight or absent. However, it was possible to show that increased pericardial pressure when accompanied by an excessive fall in blood pressure caused a reversible increase in muscle tone.

SUMMARY

1. Hemorrhage leads in unanesthetized, decerebrate dogs to a rise in muscle tone which is reversed on reinfusion of the blood.

2. Adrenalin raises blood pressure and muscle tone but ephedrine is without effect on the muscle tone in concentrations which have a decided pressor effect.

3. Hemorrhage causes a rise in muscle tone even when a fall in blood pressure is prevented by simultaneous injection of ephedrine.

4. Bilateral denervation of the carotid sinus area does not prevent the rise in muscle tone during hemorrhage.

REFERENCES

BEIGLBOCK, W. AND K. STEINLECHNER. Verh. dtsch. Ges. Kreisl Forsch. p. 301, 1938.

BRUNER, H. AND W. MERTENS. Arch. Kreisl Forsch. **3**: 223, 1939.

GELLHORN, E. AND F. POLLACK. Unpublished observations.

HENDERSON, Y. Adventures in respiration. Baltimore, 1938.

HENDERSON, Y., A. W. OUGHTERSON, L. A. GREENBERG AND C. P. SEARLE. This Journal **114**: 261, 1936.

KOCH, E. Die reflektorische Selbststeuerung des Kreislaufes. Dresden and Leipzig, 1931.

SCHLOSSBERG, T. AND M. E. M. SAWYER. This Journal **104**: 195, 1933.

EFFECT OF LOWERED OXYGEN TENSION OF INSPIRED AIR ON THE RESPIRATORY RESPONSE OF NORMAL SUBJECTS TO CARBON DIOXIDE[1]

NATHAN W. SHOCK AND MAYO H. SOLEY

From the Division of Physiology, the Departments of Medicine and Pharmacology, University of California Medical School, Berkeley and San Francisco

Numerous investigators have shown that tolerance to lowered oxygen tensions is increased by the addition of 3 to 6 per cent CO_2 to the inspired air (1, 2, 4, 5). In these experiments in which three or four experimental subjects were studied, individual differences in the physiological responses were noted (1, 2). Experiments on animals have led to the conclusion that the stimulating effects of increased CO_2 and lowered O_2 in inspired air on respiration are additive (1, 2) except when the oxygen tension falls extremely low (3). It has also been shown that the respiratory response to 1 to 4 per cent CO_2 is increased at high oxygen tensions (6). The following experiments were planned to investigate individual differences in respiratory response of normal humans to administration of carbon dioxide when the oxygen tension is diminished.

Eighteen male college students, ranging in age from 18 to 26 years, served as subjects. All were familiarized with the procedure by a trial experiment and were then tested twice with each of the following gas mixtures: *a*, 2 per cent carbon dioxide with 21 per cent oxygen; *b*, 2 per cent carbon dioxide with 17 per cent oxygen, *c*, 2 per cent carbon dioxide with 12 per cent oxygen; *d*, 17 per cent oxygen; and *e*, 12 per cent oxygen. The experimental procedure and methods of data analysis have been described previously (6).

Experimental results are shown in table 1. The average resting respiratory volume (corrected to standard conditions) in ten experiments on each of the 18 subjects was 6.89 liters per minute, as compared to 6.92 liters per minute found in previous experiments (6). Inhalation of 17 per cent oxygen caused no significant change in average respiratory volume, while 12 per cent oxygen caused an average increment of 10.6 per cent. Inhalation of 2 per cent carbon dioxide mixed with 21 per cent oxygen increased the respiratory volume 28 per cent on the average, a figure that does not differ significantly from the increase of 34 per cent found previously (6) or from that of 31 per cent caused by breathing 2 per cent carbon dioxide mixed with 17 per cent oxygen (C.R. 1.7). Inhalation of 2 per cent carbon dioxide mixed with 12 per cent oxygen resulted in an increase of 38.9 per cent in the average respiratory volume which is sig-

nifieantly greater than the increase caused by inhalation of 2 per eent carbon dioxide in 21 per cent oxygen (C.R. 2.9).

The arrangement of the experiment made it possible to compare for eaeh subjeet the actual respiratory change induced by 2 per cent earbon dioxide in a given oxygen eoneentration with the ealeulated increment in respiratory volume based on the effect of 2 per cent earbon dioxide at normal oxygen tension and

TABLE 1

Per cent change in resting respiratory volume produced by inhalation of gas mixtures

SUBJECT NUMBER	2% CO₂ IN 21% O₂			2% CO₂ IN 17% O₂			2% CO₂ IN 12% O₂			17% O₂			12% O₂		
	Test I	Test II	Av.	Test I	Test II	Av.	Test I	Test II	Av.	Test I	Test II	Av.	Test I	Test II	Av.
1	24	29	27	33	41	37	80	107	94	6	4	5	20	39	30
2	30	43	37	43	32	38	35	33	34	3	7	5	—4	—6	—5
3	58	26	42	63	44	54	45	45	45	—2	10	4	26	13	20
4	22	34	28	27	26	27	21	45	33	13	18	16	10	14	12
5	42	25	34	23	21	22	50	41	46	2	2	2	0	16	8
6	30	31	31	66	47	57	35	37	36	6	1	4	7	13	10
7	38	13	26	36	26	31	40	32	36	1	—6	—3	3	—4	—1
8	26	28	27	36	23	30	45	38	42	—3	—2	—3	18	16	17
9	25	24	25	29	31	30	47	45	46	—8	—5	—7	—6	3	—2
10	32	28	30	25	34	30	35	38	37	3	1	2	17	8	13
11	28	12	20	26	16	21	34	36	35	—6	0	—3	17	24	21
12	15	23	19	26	16	21	32	29	31	—2	1	—1	9	10	10
13	35	39	37	26	33	30	40	45	43	5	1	3	8	18	13
14	27	21	24	27	20	24	29	11	20	—3	5	1	12	9	11
15	29	24	27	32	19	26	37	27	32	—10	—3	—7	8	1	5
16	21	22	22	31	30	31	15	12	14	—8	—11	—10	14	8	11
17	19	13	16	18	30	24	40	35	38	—3	—4	—4	6	—1	3
18	31	25	28	27	23	25	35	41	38	—3	0	—2	11	18	15
Mean	29.6	25.6	27.8	33.1	28.4	31.0	38.6	38.7	38.9	—0.5	1.1	0.1	9.8	11.1	10.6
σd	12.1	10.3	9.5	15.1	11.4	12.6	16.4	21.9	18.6	5.9	6.5	5.9	8.6	11.1	9.1
σMn	2.8	2.4	2.2	3.6	2.7	2.9	3.9	5.2	4.4	1.4	1.5	1.4	2.0	2.6	2.1
C.R.*		10.4	10.5	12.4	9.3	10.5	10.4	10.0	7.5	8.9	0.3	0.7	0.1	4.8	4.2	5.0

* C.R. (critical ratio). Mean difference $/\sigma_{\text{mean difference}}$.

Where values for C.R. are less than 2.5, the administered gas mixture had no significant effect on mean respiratory volume.

the respiratory effect of the lowered oxygen tension in the absence of carbon dioxide. On the average, the computed and the experimentally obtained values agree within the errors of measurement. (For 12 per cent oxygen the ealeulated increment in average respiratory volume is 38.4 per cent, while the experimentally observed value is 38.9 per cent.)

Examination of table 1 brings to light wide individual differences in respiratory response to both carbon dioxide increase and oxygen lack in the inspired

air. Although the average values offer evidence that oxygen lack and carbon dioxide increase have an additive effect on respiration, individual subjects are found in which a reduction of the oxygen content of the inspired air reduces the respiratory response to carbon dioxide (see subjects 3, 14 and 16). In contrast to these subjects, others were found in which the respiratory response to 2 per cent carbon dioxide was significantly increased over that anticipated from simple summation of response to 2 per cent carbon dioxide and a decrease of oxygen content of inspired air to 12 per cent administered separately. Although 17 per cent oxygen did not produce a significant change in average respiratory volume for the group, one subject (no. 4) showed a measurable increase. On the other hand, five of our subjects (nos. 2, 7, 9, 15, 17) showed no significant change in respiratory volume when the oxygen content of inspired air was reduced to 12 per cent, although the average increase for the group was 10 per cent.

Thus it is apparent that individuals differ significantly in their respiratory response to carbon dioxde and lowered oxygen tension in the inspired air. It is also shown that while average values indicate that respiratory effects of increased carbon dioxide and lowered oxygen are additive in humans as well as animals (2, 5) there is no assurance that every individual subject will show the same tendency. It is also apparent from our experiments that the oxygen tension must be reduced below 91 mm. in inspired air before carbon dioxide begins to lose its efficacy as a respiratory stimulant.

SUMMARY

The respiratory response to 2 per cent carbon dioxide mixed with 21 per cent, 17 per cent and 12 per cent oxygen was measured in 18 male college students. The respiratory response to 17 per cent and 12 per cent oxygen alone was also determined. It was found that the average respiratory increment observed from the administration of a carbon dioxide-oxygen mixture could be estimated by the simple addition of the average effect of the two gases administered separately. However, marked individual differences in respiratory response were found which make predictions of the respiratory response of an individual subject to any of the gas mixtures used, impossible. Reduction of the oxygen content of the inspired air to 17 or 12 per cent does not decrease the respiratory response to carbon dioxide.

REFERENCES

(1) Asmussen, E. and H. Chiodi. This Journal 132: 426, 1941.
(2) Dill, D. B. and N. Zamcheck. This Journal 129: 47, 1940.
(3) Eastman, N. J. Am. J. Obstet. and Gynec. 31: 563, 1936.
(4) Gellhorn, E. Am. J. Psychiat. 93: 1413, 1937.
(5) Schmidt, F. and J. H. Comroe, Jr. Ann. Rev. Physiol. 3: 151, 1941.
(6) Shock, N. W. and M. H. Soley. This Journal 130: 777, 1940.

BODY TEMPERATURE OF MICE DURING ANESTHESIA

E. F. BARROWS AND H. DODDS

From the Oregon College of Education, Monmouth

Received for publication March 30, 1942

The ability of day-old mice to survive ether vapor for more than ten times the length of exposure which would kill normal healthy adults was discussed by one of the present authors in an earlier paper (Barrows, 1933). Also Avery and Johlin (1932) had noted somewhat similar results with illuminating gas, carbon monoxide, nitrogen, argon or hydrogen. It was our opinion that a fall in the internal temperature of the young mice would account for practically all of this slower effectiveness of the anesthetic. To test this opinion we have now made several series of further experiments.

First, six series of experiments had ether as the anesthetic; and of these six, two were at 22°C, two at 35°C and two at 40°C, on the theory that the warmer the environment, the less the young mouse's internal temperature would fall, and hence the sooner it would die. At each of these three temperatures one of the two series employed wild-type gray mice (labeled *Gr* in fig. 1), and the other employed non-agouti brown dilute mice (labeled *Dil* in fig. 1). Each strain was from the Roscoe B. Jackson Memorial Laboratory and had been long inbred to produce high genetic uniformity.

Second, four more series of experiments had chloroform[1] as the anesthetic, one series at 22°, one at 28°, one at 35° and one at 40°C.

It was known that new-born mice were not able to maintain a uniform body temperature against external changes, but only acquired homoeothermal ability gradually during the first two or possibly three weeks after birth (Sumner, 1913). Thus the younger the mouse below about its third week, the more readily its internal temperature could be lowered by a cooler environment. And of course the cooler the environment, the greater the drop in the mouse's internal temperature. If, on the other hand, the environment were kept warm enough, there would be no drop in the internal temperature at any age, and so all mice should die at the same rate.

An examination of figures 1 and 2 will show to what extent our expectations were realized. The temperatures given were of the part of the flask where the mouse lay, about one degree lower than the actual thermometer readings.

PROCEDURE. The apparatus was: several 3-liter flasks with rubber stoppers each holding a long thermometer projecting into the lower center of the flask; a heating chamber in which the temperature of the flask could be controlled and observed; anesthetic ether; anesthetic chloroform; watch; scales; graduated pipette.

The liquid anesthetic (2.4 cc. of ether or 1.0 cc. of chloroform) was introduced

---
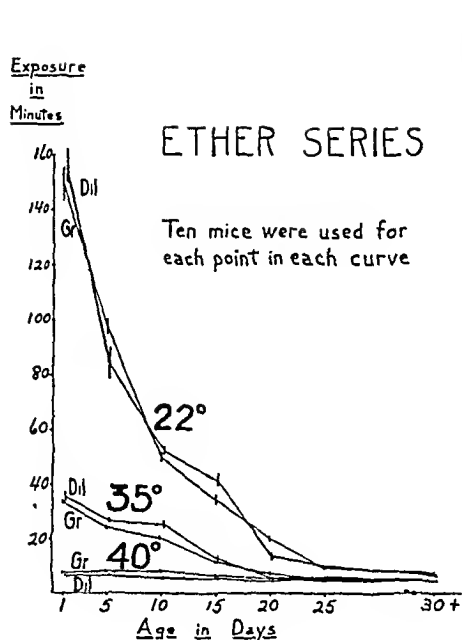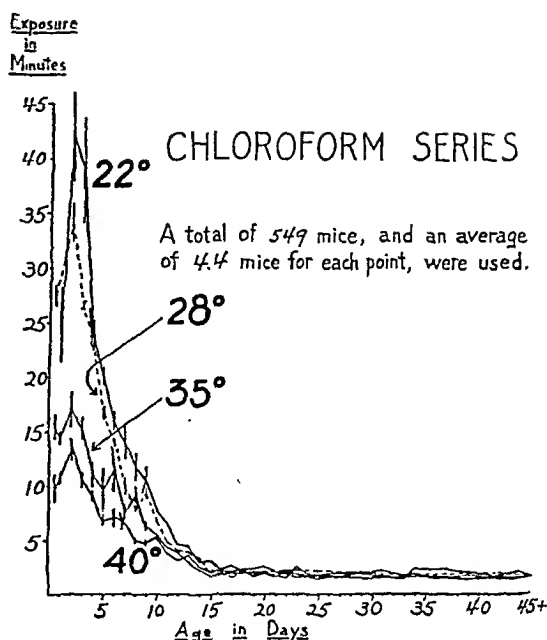
Fig. 1   Fig. 2

Fig. 1. This figure shows the number of minutes required (ordinates) for a certain concentration of ether vapor to cause the death of mice of different ages (abscissas). Three flask-temperatures were used, 22°, 35° and 40°C. At each temperature two series were run, one using wild-type gray mice (marked *Gr*), and one using non-agouti brown dilute mice (marked *Dil*). There was evidently no significant difference in the reactions of the two strains; for this reason we did not use the grays in the chloroform series (shown in fig. 2).

In order to make the reading of these curves easier, the age-points for the "dilutes" have been displaced slightly to the right. As a matter of fact, these points are probably a more, rather than less, accurate index of the age of these mice, as in most cases a litter was born the night before the day recorded as its "birthday." Mice are generally born at night, most often between midnight and 4 a.m. (Merton, 1938).

We have as yet no explanation for the fact that both of the 22° curves run consistently higher than the others at the 20-day age and over.

The vertical lines show the calculated standard error of the mean for each group.

Fig. 2. This figure shows the number of minutes required (ordinates) for a certain concentration of chloroform vapor to cause the death of mice of different ages (abscissas). We used the same three flask temperatures as in the ether series, and also one additional, 28°C., indicated by the broken line. We also here determined points at one-day rather than five-day intervals.

The vertical lines, showing the magnitude of the standard error of the mean at each point, were omitted at points from the tenth day on, to avoid confusion. These omitted lines in the 10-day to 15-day ages had an average length of less than 0.9 minute ($S_{\bar{x}}$ = less than 0.45 min.); and above the 15-day age, an average length of about 0.3 minute ($S_{\bar{x}}$ = about 0.15 min.). A few of the lines for before the 10-day-age had to be displaced slightly to right or left to avoid contact with other lines, but we believe that these cases will be clear to the reader as he comes to them.

In these chloroform series, the observations at 22°, 28° and 40° were by Pearl Clauson; those at 35° by Flora Lee Bertsch.

into the flask and the stopper kept loosely in place until vaporization was complete. Then with the stopper still in place, the flask was inverted for several seconds, and then allowed to stand in the heating chamber under close

observation until the required temperature was reached inside the flask.   There-upon the mouse was inserted into the neck of the flask and allowed to slide rather than drop to the bottom; the stopper was at once replaced and the time recorded.   As soon as he became quiet he was rolled over, by turning the flask, in order to allow better observation of the breathing movements.   The last observed movement of any kind (the assumed death point) was nearly always a contraction of the diaphragm.   The time of the final movement was recorded, the flask temperature having been kept carefully at the same level all this while.   The mouse was then removed and weighed.

DISCUSSION.   The two figures set forth in some detail the evident correlation of time of death under ether (or chloroform) with both age and environmental temperature in young mice thereby supporting the theory that the slower death of these animals is due to their lower internal temperature.   The reader is referred to the ten curves themselves as the most effective presentation of this main point.

But some minor peculiarities of the curves should receive mention:

1. At 40° the two ether curves are almost flat but the chloroform curve is markedly higher for the earlier ages.

2. In the chloroform series, mice one day old or less reversed the general trend: that is, they died *sooner* than the two-day-olds.   No two-day-olds were tested in the ether series.

3. We found no evidence in mice of the five abrupt, discontinuous steps reported by Cameron (1941) and considered by him as indicating five abrupt changes in the respiratory chemistry of the rats and rabbits used in his experiments.   However, the anesthetic which he employed (CO), rather than the use of other species, may account for this difference.

In conclusion, we believe that these curves indicate the possible value to surgical technique of combining an anesthetic with a lowered body temperature, thus increasing the length of the narcosis and anesthesia time, without increasing the amount of the anesthetic which the body receives.

SUMMARY

1. With mice more than three weeks old, differences in age or in environmental temperature are not effective in changing the length of time required to kill them with ether or chloroform.

2. But with mice less than three weeks old, the effectiveness of these anesthetics diminishes as younger and younger mice are tested.

3. And also with mice less than three weeks old, the effectiveness of these anesthetics diminishes as the mice are exposed to colder environmental temperatures.

4. These observations support the theory that a fall in the internal temperature is the principal cause of the slower effectiveness of the anesthetics.

5. A possible application to surgery is suggested.

6. Two homozygous strains of mice differing from each other in three unlinked pairs of genes, show no significant differences in their times of succumbing to ether.

REFERENCES

AVERY, R. C. AND J. M. JOHLIN. Proc. Soc. Exper. Biol. and Med. **29**: 1184, 1932.

BARROWS, E. F. Proc. Soc. Exper. Biol. and Med. **30**: 1290, 1933.

CAMERON, J. A. J. Cellular and Comp. Physiol. **18**: no. 3, 1941.

MERTON, H. Proc. Royal Soc. Edinburgh **58**: 80, 1938.

SUMNER, F. B. J. Exper. Zool. **15**: 315, 1913.

THE ELECTRIC RESPONSES OF THE TAIL PILOMOTORS AND NICTITATING MEMBRANE OF THE CAT

A. ROSENBLUETH AND E. C. DEL POZO

With the assistance of G. H. ACHESON

From the Department of Physiology in the Harvard Medical School

Since Rosenblueth, Leese and Lambert (1933) described the electric responses of some smooth muscles in the cat, several other studies on this problem have appeared. Neither the systematization, nor, particularly, the interpretation of these electric phenomena is uniform. The data suggest that some of the discrepancies are due to the fact that although smooth muscles form a homogeneous anatomical group, physiologically the group is probably heterogeneous (see Rosenblueth, 1941).

The purpose of this study was to compare the electric responses of two smooth muscles of the cat, the nictitating membrane and the tail pilomotors. These two muscles were selected because of their similarity and simplicity: both consist of oriented elements, readily available for study; both have a single excitatory adrenergic sympathetic nerve supply; both respond to single shock stimulation; both exhibit little or no spontaneous activity.

METHOD. Cats were used, under dial anesthesia (Ciba, 0.7 cc. per kgm., intraperitoneally). For recording from the nictitating membrane the lids were separated at the outer canthus and the eyeball was removed after severance of the conjunctiva and the extrinsic striated muscles. The mechanical tracings were obtained by attaching the free end of the membrane to a light Sherrington frictionless torsion-spring myograph. The beam of light reflected by the mirror on the myograph was sent to the back of the film in the camera that photographed simultaneously the electrograms from a cathode ray oscillograph.

A mechanogram which reflected closely pilomotor activity was obtained as follows. Contraction of the pilomotor muscles results not only in erection of the hairs, but also in a movement of the skin toward the base of the tail. A small transverse cut was made and a serrefine was clipped on the basal flap of the wound. This serrefine was attached to a torsion-spring myograph and the movements of the skin were photographed as in the case of the membrane. Careful simultaneous observations of the erections of the hairs in response to sympathetic stimulation and of the excursions of the beam of light from the myograph showed that the movements were quite parallel.

The electric responses were led off the animal by means of wicks moistened with Ringer and connected to impolarizable chlorided silver plates. The position of the leads varied in different experiments and will be described with the results. The responses were amplified usually by a 5-stage direct-coupled amplifier and recorded from a cathode-ray oscillograph. Exceptionally capacity-coupled amplification was used.

The stimulating electrodes were silver wires, insulated by rubber from surrounding tissues. They were placed on the preganglionic cervical sympathetic trunk in the neck, after central section, or on the lumbar chains, at the level of L4, after central crushing. The stimuli were condenser discharges through a thyratron.

The injections of cocaine, veratrine, ergotoxine and piperidinomethyl-3-benzodioxane (933F) were made intra-arterially, into the carotid for the membrane and into the abdominal aorta for the pilomotors of the tail. When veratrine was injected into the carotid the brain was previously pithed, to prevent the marked and persistent contractions of the striated musculature of the head, which otherwise ensue.
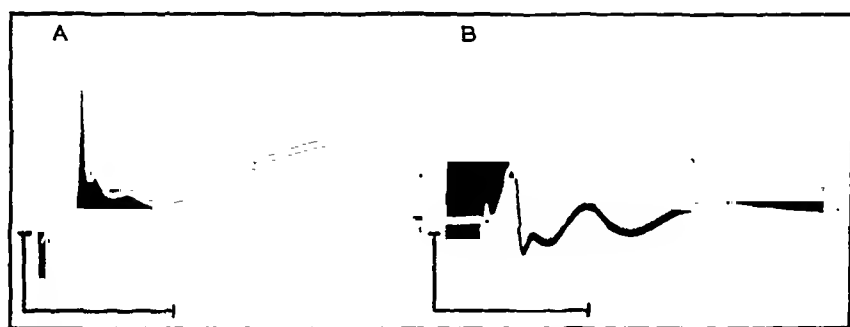
Fig. 1. Normal electrograms. In this and the following figures the records were taken with a direct-coupled amplifier, unless otherwise stated. The small, sharp excursion which sometimes precedes the response is the stimulus artifact. Maximal stimuli were employed. The amplitude of the responses and the speed of the record are calibrated by the lines in the left lower corner.

A. Pilomotors of the tail. The arrow indicates the time of stimulation. Calibrations: 1 sec. and 1 mv. In this and the following pilomotor records the leads were placed on subcutaneous tissues, about 5 cm. apart, near the terminal region of the tail, after removal of a small portion of the skin at each lead. Upward excursions denote positivity of the electrode placed toward the base of the tail with respect to the one placed toward the tip.

B. Nictitating membrane. Leads at the free edge and the external canthus, respectively. Upward excursions denote positivity of the free edge. Calibrations: 0.5 sec. and 0.2 mv.

RESULTS.   A. *The Pilomotors of the Tail.*   a. *Responses to single maximal shocks.* A characteristic electrogram from the tail is illustrated in figure 1A. The response is to a single maximal shock applied to the lumbar sympathetic chains. The lead-off electrodes were placed lengthwise, about 5 cm. apart, in contact with subcutaneous tissue, after removal of about 1 sq. cm. of skin at each contact.

Figure 1A is labeled "pilomotor response." That structures in the tail other than the pilomotors do not contribute to the electrogram was shown by the following controls. Removal of the skin between the leads abolished all electric responses (cf. Rosenblueth, Davis and Rempel, 1936). If the leads were placed transversely on the tail, instead of longitudinally, all electric responses disappeared; this proves that the elements responsible for the electric phenomena are specifically oriented in the tail and the only oriented structures known in the

skin are the pilomotors. The possible influence of vasomotor changes on the electrograms was investigated by comparing normal responses with those obtained after clamping the abdominal aorta; no significant difference was seen. Finally, in the dog, stimulation of the lumbar sympathetics leads to pilomotor erection in the base of the tail, but not toward the tip; in 2 dogs electric responses, quite similar to those in the cat, were seen when the leads were at the basal region, where erection of the hairs was observed, and no electric phenomena appeared when the leads were placed near the tip.

The electrogram in figure 1A is complex. Lambert and Rosenblueth (1935) recognized three components of the pilomotor electric response, but that appears now to be too simple a systematization. In the record there are 7 successive swings of alternating polarity—i.e., the spot moves first in the direction indicative of positivity of the base, then in the opposite direction, etc. A systematization into 7 components is thus suggested. It is possible, however, that fewer than 7 components could yield 7 waves. Thus, some of the components could be diphasic, or else a brief excursion of a given polarity could break into a longer
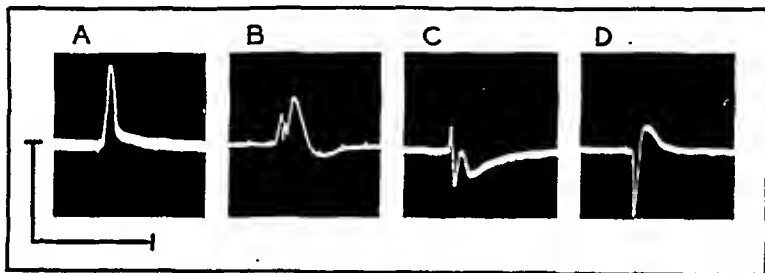
Fig. 2. Different types of normal initial responses from the pilomotors. Calibrations: 2 sec. and 0.5 mv. The records are from different animals; the leads were placed similarly. From A to D component *1* decreases, while component *2* increases.

excursion of opposite sign if it were superimposed in its course. Since the 7 swings in figure 1A were often observed, however, with approximately similar latencies (fig. 6B), reference will be made to them as waves *1* to *7* of the electrogram, respectively. The evidence presented below will identify some of these swings as individual, independent components.

b. *Components 1 and 2.* The initial excursion of the electrogram was usually a sharp swing in which the base became positive with respect to the tip. Occasionally, however, a base-negative swing started the response. In figure 2 are shown a series of records from different animals illustrating pure base-positive (A), diphasic (B and C) and finally pure base-negative (D) initial excursions.

This apparent inconsistency of the early swing of the electrogram had been noted by Rosenblueth, Davis and Rempel (1936). The explanation suggested now is that there are two early successive monophasic components, of opposite polarity, *1* base-positive, and *2* base-negative. In some animals component *2* is entirely masked by *1* (fig. 2A); in others, *1* is minimal or absent (fig. 2D). In some experiments an apparent reversal of the initial excursion of the electrogram took place (cf. Rosenblueth, Davis and Rempel). Invariably in such cases, if

the reversal was from base-positive to base-negative the latency of the responses was lengthened (transition from *1* to *2*), and conversely, if the reversal was from base-negative to base-positive the latency decreased (appearance of *1*).

In a series of observations the responses to single shocks were recorded before and after a brief period of rapid stimulation. Characteristically the post-tetanic responses showed a large increase of the mechanogram and several changes of the electrogram. Prominent among the latter was a marked increase of component *2* at the expense of *1*. Figure 3 illustrates an observation taken with a capacity-coupled amplifier in order to filter out the large component *7* which develops upon repetitive stimulation (see below). The increase of *2* after the tetanus and the gradual return of *1* are typical.

An independent increase of either *1* or *2* was seen after injections of drugs. Thus, in figure 4A, component *1* increases in the course of repetitive stimulation
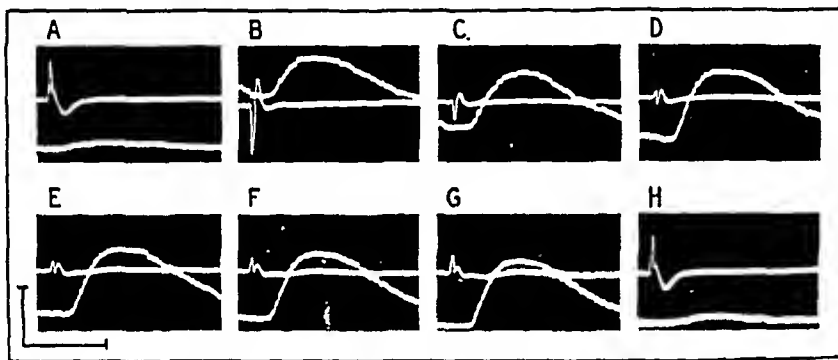
Fig. 3. Post-tetanic increase of the mechanical response and of component *2* of the pilomotors. Capacity-coupled amplifier. Calibrations: 2 sec. and 2 mv. Responses to single shocks applied as follows. A: control before tetanic stimuli. B to G: 15, 22, 29, 36, 43 and 50 sec., respectively, after 2-sec. stimulation at the rate of 60 per sec. H: control 5 min. later. In this and other figures illustrating mechanical responses of the pilomotors, the myograph (lower tracing) was attached to the skin. See Method for the explanation of this procedure.

after cocaine, while in figure 4B a similar increase of *2* is illustrated after 933F. A striking separation of the two components also occurred after injections of ergotoxine. Shortly after the injection component *1* increased up to 150 per cent of its original amplitude. It later decreased until total disappearance. Component *2* was still present, however, and increased markedly with repetitive stimulation (fig. 5).

c. *The swings 3 to 6.* The inference that the excursions *1* and *2* denote separate, independent components of the electrogram is justified by the data presented above (figs. 2 to 5). A similar inference for the swings *3* to *6* does not emerge as clearly from the observations. Thus, the swing *3* might be a continuation of component *1*, transiently masked by a brief *2*. Or else *4* and *5*, which frequently varied together and which followed each other without any obvious discontinuity, could be a single diphasic component.

A careful study of the records, however, suggests that all the excursions under

consideration are capable of independent variation in different experimental conditions. A detailed description of the behavior of each of these swings in the several experiments would be lengthy and unfruitful; a few observations showing the preponderance or absence of some of them are illustrated in figures 6 and 7. It is tentatively concluded that the swings *3* to *6* denote the development of separate components of the electrogram.

d. *Component 7*. The final slow base-positive wave of the electrogram appeared in all the animals studied. In some cases it was not obvious after single-
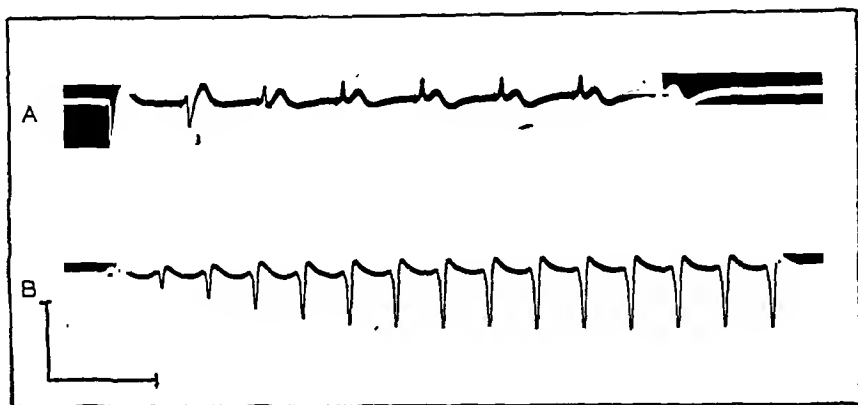
Fig. 4. Increase of component *1* or *2* of the pilomotors upon repetitive stimulation. Calibrations: 2 sec. and 2 mv.

A. After cocaine (6 mgm. per kgm.). Component *1* increases progressively. B. In another animal, after 933F (5 mgm. per kgm.). Component *2* increases progressively.
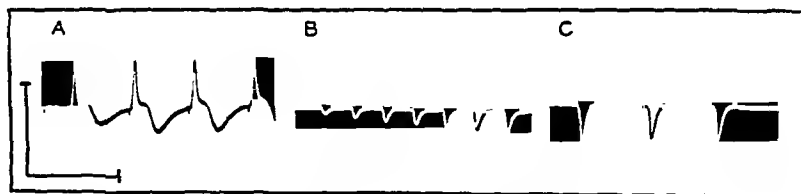
Fig. 5. Disappearance of component *1* and persistence of component *2* of the pilomotors after ergotoxine (1 mgm. per kgm.). Calibrations: 2 sec. and 2 mv.

A. Responses before ergotoxine. B. Responses 15 min. after ergotoxine. Component *1* increased immediately after the injection of the drug and later disappeared. C. Responses 5 min. later. The increase of component *2* is due to an intervening tetanic stimulation.

shock stimulation, but it then emerged clearly upon the delivery of 2 to 7 shocks at relatively slow frequencies (1 to 4 per sec.).

That this slow wave belonged to the pilomotor electrogram was shown as follows. As already mentioned (section *a*) the wave disappeared upon transverse, instead of longitudinal, recording. Removal of the skin between the leads also abolished the response. Clamping the aorta did not prevent its development. It was absent at the tip of the tail of the two dogs studied, and present at the base. Spurious slow waves could be due to a change of resistance between the leads if the direct-coupled amplifier were unbalanced. But such

spurious waves would change in polarity with the direction of the unbalance in the amplifier. The wave 7, however, always indicated positivity of the base with respect to the tip of the tail. Spurious waves could also result from a shift of contact of the leads with the tissues (see nictitating membrane, below). Since the skin moves with the pilomotor contractions such shifts of contact are possible. But in the majority of the observations the leads were in contact with the subcutaneous tissues and enough skin was removed so that no movement could affect this contact. Neither vasomotor activity nor spurious changes would probably be significantly affected by the interelectrodal distance, yet component 7 was found to increase with the interelectrodal distance within a range of 1 to 6 cm. The other components of the electrogram also increased
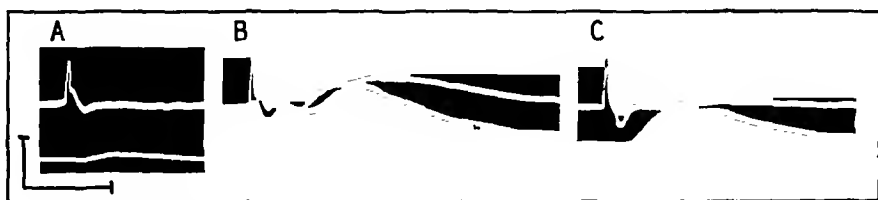
Fig. 6. Independent growth of component 4 of the pilomotor electrogram. Calibrations: 2 sec. and 2 mv.

A. Control before cocaine. B and C. Responses to two shocks 12 sec. apart after cocaine (3 mgm. per kgm.). Component 4 increases in the second response, while components 6 and 7 decrease.
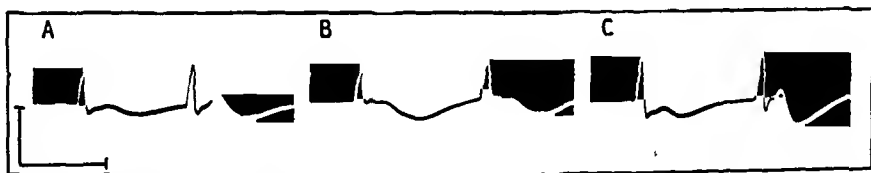
Fig. 7. Independence of components 5 and 6 of the pilomotor electrogram. Calibrations: 1 sec. and 2 mv. Responses to two shocks.

A. Normal control. The second shock elicits a large component 5. B. Three minutes after tetanic stimulation. The electrogram consists mainly of components 1 and 6. C. After veratrine (0.5 mgm. per kgm.). The second shock elicits a large component 6.

with the distance between the electrodes (cf. Rosenblueth, Davis and Rempel, 1936).

The amplitude and duration of component 7 were directly proportional to the number and to the frequency of the shocks applied. In figure 8 the influence of the number of shocks is illustrated. With high frequency stimulation (e.g., 30 per sec.) component 7 could attain 5 times the amplitude of component 1, it could thus be as large as 5 mv. and its duration up to several minutes.

e. *Relations between the electrogram and the mechanogram.* The behavior of the several components of the electrogram was studied in responses in which the amplitude of contraction of the pilomotors was modified by different experimental conditions. This analysis revealed that the early components 1 to 5 did not show any correlation with the amplitude of contraction.

Thus, after cocaine the mechanical responses to single shocks were markedly increased (cf. Rosenblueth and Rioch, 1933), and component *1* was only slightly larger than normal. If several stimuli were delivered at a rate of 5 to 20 per min., the contractions gradually declined in amplitude, while *1* remained large (fig. 6B and C). Similarly, after ergotoxine the initial increase of *1* coincided with a decrease of contraction.

The independence of *2* and of the mechanical responses was indicated by the effects of ergotoxine and of 933F. With the first drug long series of stimuli could be delivered at a slow rate which caused electric responses consisting mainly of a large *2* (see fig. 5) and with little or no erection of the hairs. After injections of 933F component *2* was usually large, while the mechanical responses were smaller than normal. Without drugs, during the post-tetanic period, marked changes of *1* and *2* took place which were not paralleled by the mechanogram (fig. 3).

With regard to components *3*, *4* and *5* it is sufficient to state that only occasionally did they show variations parallel with the changes in contraction.

In contrast with the waves *1* to *5*, components *6* and especially *7* frequently changed in amplitude as did the mechanical responses. Thus, they were larger than normal after injections of cocaine (fig. 6) or veratrine (fig. 7C), drugs which increase the mechanical effects of single shocks; and they were smaller or absent after 933F (fig. 4B) or ergotoxine (fig. 5), drugs which decrease the mechanical responses.

The time course of *6* and *7* cannot be determined accurately, since there is no indication of their precise beginning and end, and since several of the components of the electrogram probably overlap. Contraction starts approximately at the beginning of *5*. In general, with the exception of the cases where a large *7* overwhelmed *6* (fig. 9C, 2nd response), the time course of *6* was similar to that of the development of tension, the peak of *6* coinciding with the middle of the rising phase of the mechanogram (fig. 9A, and C, 1st response).

The time course of *7* was invariably delayed as compared with that of the mechanogram. Thus the peak of *7* could appear late during the period of relaxation, and total relaxation was often seen while *7* was still of measurable amplitude (figs. 6 and 9).

f. *The resting potential.* A difference of potential was found consistently between the leads when they were placed longitudinally on the tail. The basal electrode was positive with respect to the tip electrode. The difference of potential increased with the interelectrodal distance at approximately 2 mv. per cm., for distances from 1 to 6 cm. Removal of the skin at one or both of the contacts did not modify the potential difference, nor did the clamping of the aorta.

Since this resting potential did not disappear when all the skin was removed between the leads it is inferred that the potential is not of pilomotor origin. Its study was therefore not pursued.

g. *The action of the drugs.* The drugs were used merely as tools for the separation of the components of the electrogram and for the examination of the rela-

tions between the electrogram and the mechanogram. For this reason the emphasis has been placed on the responses, not on the effects of the drugs. In this section is given a summary of the actions seen.

Cocaine (3 to 8 mgm. per kgm.) caused a marked increase of the pilomotor mechanical responses to single nerve volleys. Several of the components of the electrogram, particularly *7*, were usually larger than normal after cocaine. The responses to shocks spaced at about 5 to 10-sec. intervals showed a progressive decrease of the mechanogram and the electric components *5, 6* and *7*; the components *1* and *4*, on the other hand, did not decline significantly (fig. 6).

Veratrine (about 0.1 mgm. per kgm.), like cocaine, caused an increase of the mechanical responses. The increase in this case, however, was probably due to repetitive discharges of the nerve fibers in response to single shocks (see Rosenblueth and del Pozo, 1942). Some or all the components of the electrograms were increased in the different experiments. In nerve axons (Graham, 1930), in the superior cervical ganglion (Rosenblueth and del Pozo, 1942), and in striated muscle (Rosenblueth, Wills and Hoagland, 1941), veratrine augments the spike potential and especially the negative after-potential. Repetitive stimulation of these structures (see also Acheson and Rosenblueth, 1941) leads to striking and characteristic changes of the responses. No specific component of the pilomotor electrogram increased after veratrine in a fashion suggestive of being analogous to the negative after-potential, nor did the changes seen upon repetitive stimulation parallel those which occur for the spike and the negative after-potential.

After injections of 933F the mechanical pilomotor response was decreased. The most striking change in the electrogram was an increase of component *2*. Repetitive stimulation led to a further increase of this wave (fig. 4B).

Ergotoxine (0.5 to 3 mgm. per kgm.) resulted in a relative paralysis of the muscles. The mechanical responses to single shocks or to repetitive trains at slow frequencies (0.5 to 5 per sec.) were readily and entirely abolished. Regardless of the dose of ergotoxine administered, however, sufficiently rapid stimulation of the sympathetic (20 to 60 per sec.) invariably caused erection of the tail hairs, the more marked the more frequent or prolonged the stimuli. It appears as if a critical concentration of sympathin has to be attained, after injections of the drug, in order to elicit muscular contraction. Some of the changes in the electrogram were described on p. 269 and illustrated in figure 5. In addition, components *3* to *7* were absent in the responses to single shocks. With frequencies sufficient to cause contraction, components *6* and *7* reappeared in the electrogram.

B. *The nictitating membrane.* a. *Responses to single maximal shocks.* The electric responses of the nictitating membrane, first described by Rosenblueth, Leese and Lambert (1933), have been further studied by Bacq and Monnier (1935), Monnier and Bacq (1935), Lambert and Rosenblueth (1935), Rosenblueth, Davis and Rempel (1936), and Eccles and Magladery (1937a and b). The description here will be brief; it will be made as a comparison with the pilomotor electrogram and will emphasize mainly features important for the evaluation of earlier interpretations.

The electric responses of the membrane may sometimes be quite similar to those of the pilomotors (cf. fig. 1A and B); they may, however, in other cases be markedly different (figs. 10 and 11). This suggests that some components may be common to both electrograms, while others may appear exclusively in one of the two muscles.
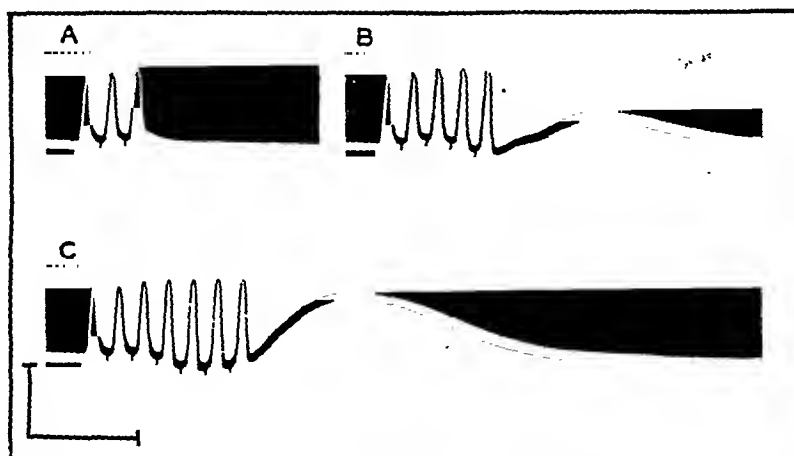

Fig. 8. Increase of component 7 of the pilomotors with the number of shocks delivered at a constant frequency. Calibrations: 2 sec. and, 1 mv. A, B, and C, 3, 5 and 7 shocks, respectively.
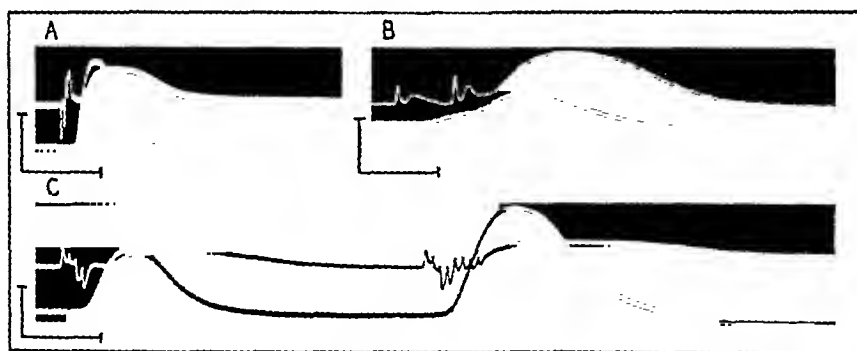
Fig. 9. Relations between the slow components of the pilomotor electrogram and the mechanogram.

A. Normal animal. Responses to 3 shocks. Calibrations: 4 sec. and 2 mv. B. In another animal, after cocaine (6 mgm. per kgm.). Calibrations: 2 sec. and 1 mv. C. In another animal. Responses to 4 and then to 8 shocks, at the same frequency. Calibrations: 2 sec. and 5 mv.

The electrogram of the membrane is strikingly influenced by the relative position of the recording leads (fig. 10E to H). In the pilomotors the position of the leads affects the relative amplitude of all the components equally (p. 264; Rosenblueth, Davis and Rempel, 1936), not differentially, as is the case for the membrane. This difference may be due to the more uniform orientation of the muscular elements in the tail than that in the membrane (Acheson, 1938).

b. *Components 1 and 2.* The initial excursion of the electrogram is sometimes

monophasic (free edge positive, fig. 10H), sometimes diphasic (fig. 10B and C). Eccles and Magladery (1937a) inferred from their observations that this initial
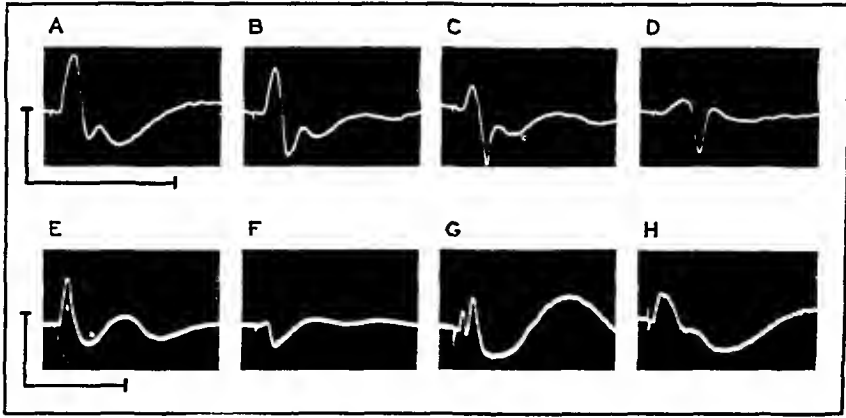

Fig. 10. Separation of components *1* and *2* in the nictitating membrane. Capacity-coupled amplifier. Calibrations: 0.5 sec. and 0.4 mv. One recording lead was placed at the free border of the membrane, the other in various positions. Upward excursions denote positivity of the lead at the free edge with respect to the second one.

A, B and C. Successive responses to shocks delivered at 1-sec. intervals. The second lead was inside the orbit, near the optic nerve. Component *2* grows at the expense of component *1*. D. In another animal. Leads as in A, B and C. Response to a single shock. In contrast with A, a small component *1* is followed by a large component *2*. E, F, G and H. In another animal. Responses to single shocks. The second lead varied as follows: E, external canthus; F, lower lid; G, upper lid; H, internal canthus. In E, component *1* is followed by *2*; F shows exclusively 2; G is an atypical complex record, a brief *2* seems to develop in the course of *1*; H shows initially a pure *1*.
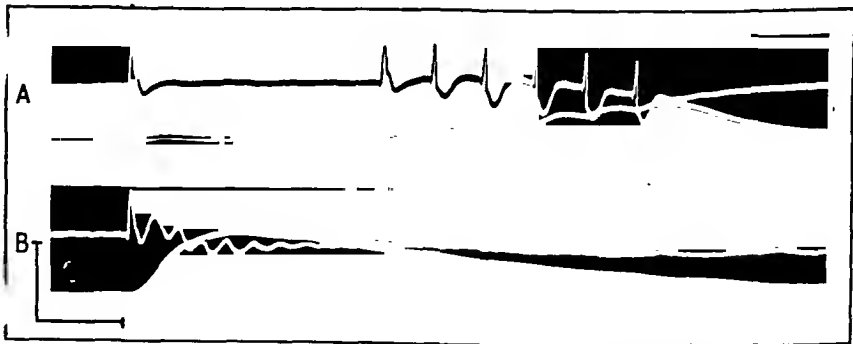
Fig. 11. Effects of cocaine (8 mgm. per kgm.) on the electrogram (upper tracing) and the mechanogram (lower tracing) of the nictitating membrane. Calibrations: 2 sec. and 0.4 mv.

A. Before cocaine. Responses, first to a single shock, then to a brief series of stimuli. B. After cocaine. Response to a single shock.

excursion was always diphasic, and that the monophasic character in some records was due to the early appearance of a later component which masked the second phase of the first diphasic wave.

The present study leads to the conclusion that in the membrane, as in the pilomotors (p. 265) two monophasic early components of opposite polarity, *1* and *2*, occasionally follow each other without a break, and suggest thus a single diphasic wave. The evidence for this conclusion is similar to that presented for the pilomotors. An independence of the relative amplitude of the two components was seen in several conditions, as follows.

The position of the leads could determine whether *1* or *2* was dominant in the early part of the electrogram (cf. fig. 10E to H). Repetitive stimulation at slow frequencies usually resulted in a progressive increase of *2*, while *1* was unchanged or decreased (fig. 10A to C). Finally, some of the drugs tested could differentially increase or decrease one or the other of the two components.
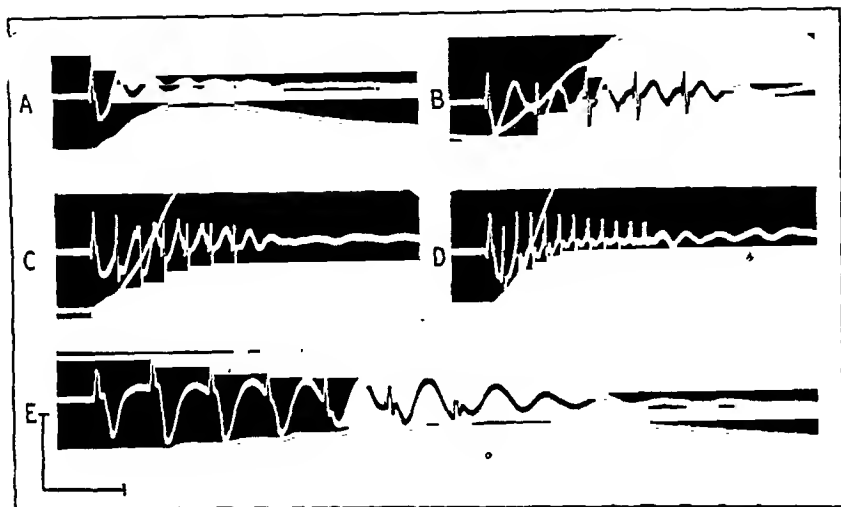
Fig. 12. Absence of refractoriness of the membrane during the course of the rhythmic waves. Calibrations: 2 sec. and 0.4 mv.

A to D, after cocaine (4 mgm. per kgm.). Responses, A, to a single shock; B, C and D, to repetitive stimuli at increasing frequencies as shown by the brief *1–2* complexes. E, in another animal, after veratrine (0.15 mgm. per kgm.). Responses to repetitive stimuli as shown by the brief component *1*.

c. *The rhythmic component.* This component (see figs. 11B and 12A) consists of a series of waves recurring rhythmically at a rate of 0.7 to 2.5 per sec. and declining gradually in amplitude. It is characteristic of the nictitating membrane; it never appeared in the pilomotors in any of the present experiments or in those previously performed in this Laboratory. It was described, but not recognized as a specific component, by Lambert and Rosenblueth (1935). It was later identified and carefully studied by Eccles and Magladery (1937b).

Our observations largely confirm those of Eccles and Magladery. In addition, it was found that injections of cocaine are very effective in reinforcing the component, when present normally, or in bringing it out, when absent. Figure 11 illustrates a typical observation. Veratrine, like cocaine, also increased the rhythmic component.

In only one point do our observations differ from those of Eccles and Mag-

ladery. They encountered evidence that the initial components in response to successive shocks were small if they developed during the course of a rhythmic wave set up by a previous stimulus. From this evidence they inferred that some of the muscular elements of the membrane were refractory to nerve impulses during a rhythmic discharge and, further, that this was proof of the fundamental similarity of the processes denoted by the initial complex and by the rhythmic waves of the electrogram, respectively.

Figure 12 illustrates series of responses of the membrane elicited at different times in the course of the development of rhythmic waves. Although occasionally components *1* or *2* seem smaller than normal this apparent decrease may well be due to the purely electrical interaction of waves of different sign. There is no clear evidence in the records, which are typical of a large series of observations, of any functional correlation between the rhythmic component on the one hand, and components *1* or *2* of the electrogram, on the other.

d. *The slow final component of the electrogram.* Rosenblueth, Leese and Lambert (1933) described a prolonged final component of the electrogram, whose beginning was roughly simultaneous with the beginning of contraction. A similar slow component, slightly preceding contraction and later following a time course parallel with that of the mechanical response, was described by Bacq and Monnier (1935). Eccles and Magladery (1937a) stated that when a slow electrical deflection was seen in their records it usually disappeared on shifting the electrodes to minimize movement artifacts.

Our experience agrees with that of Eccles and Magladery on the importance of movement artifacts as a source of error. These artifacts are difficult to avoid since rigorous isometric contractions have thus far not been achieved for the membrane, as will be shown in the discussion. Little or no movement artifacts were present, however, when one lead was placed close to the insertion of the myograph, tightened until no significant movement of that portion occurred, and the other on the periorbital tissues, e.g., at the external canthus, where little or no smooth muscle is present. Although only small, a slow late component invariably developed in these conditions upon repetitive stimulation for 1 or 2 sec. at frequencies of 5 to 20 per sec. The polarity of this component was usually free-edge positive. It may be inferred, therefore, that there probably is a slow late component of the electrogram of the membrane (see Lambert and Rosenblueth, 1935). This component may be analogous to component *7* of the pilomotors (figs. 8 and 9), but there is a striking difference in the amplitude of the two phenomena—the pilomotor wave is much larger than that of the membrane.

e. *The action of the drugs.* Some effects of cocaine on the responses of the membrane were reported by Rosenblueth and Cannon (1936). The large increase of the rhythmic component illustrated in figure 11 may be added here. Rosenblueth and Cannon also described some effects of 933F. The present observations indicate that, as in the pilomotors, the increase of the initial complex is due mainly to an increase of component *2*. Injections of this drug to normal or to previously cocainized cats resulted in a decrease or disappearance of the rhythmic component.

As already reported by Rosenblueth, Leese and Lambert (1932), ergotoxine abolishes all the electric responses of the membrane. This is in contrast with the persistence of some of the pilomotor components even after large doses of the drug (fig. 5).

Veratrine, like cocaine, increased the rhythmic component of the electrogram. As in the case of the pilomotors (p. 270) no components suggested after veratrine a similarity with the spike potential and the negative after-potential of striated muscle or of nerve.

DISCUSSION. A. *The methods.* In studies dealing with electric changes in muscle, isometric contractions are preferable to isotonic conditions, because the changes of length of the muscular elements may modify the electrogram and because they may lead to movement artifacts (p. 274). A method which would permit the isometric recording of pilomotor activity is not available. The mechanograms in figures 3, 6 and 9 merely indicate the time course and the gross amplitude of the pilomotor response.

Even in the nictitating membrane, where isometric conditions appear more readily attainable, the present and previous mechanograms are not isometric. The smooth muscle that causes withdrawal of the membrane has no insertions on bone (Acheson, 1938), so that fixation of the free edge of the membrane without a concomitant immobilization of the intra-orbital portion of the muscle does not prevent an undetermined degree of shortening.

Bacq and Monnier (1935) claimed that monophasic electric recording from the membrane, instead of diphasic, is possible if a portion of the muscle is depolarized by means of KCl. Monophasic recording would be quite desirable because it would give a more accurate picture of the changes at a given point in the cells, and because it might distinguish conducted from non-conducted events.

Bacq and Monnier (1935) and also Monnier and Bacq (1935) did not obtain monophasic recording conditions by their procedure. The KCl lead was not on muscle, but on cartilage and connective tissue covered by the conjunctiva. Indeed, a comparison of records obtained with the present "diphasic" leads, with records secured by the method of Bacq and Monnier, in the same animals, reveals no significant difference.

If the muscle elements in the membrane and pilomotors are discrete units and do not form a syncytial tissue, as is probable, then monophasic recording could only be attained by damaging a portion of an individual cell and studying that single element.

The principles involved in the previous argument apply to other aspects of the problem of the smooth-muscle electrograms. Bacq and Monnier (1935) described the measurement of a demarcation potential in the membrane by the technique mentioned. Not only is the existence of this demarcation potential questionable on the grounds already expressed but, in addition, the use of KCl for "depolarization" of tissues may be criticized as follows. As is well known, there is a difference of potential between two solutions of different concentrations, when connected electrically. The application of KCl to a certain region of a tissue may therefore set up a concentration cell, and the difference of poten-

tial measured may not denote a demarcation potential. Indeed, a KCl lead placed on the mesentery, or on a tendon, records a difference of potential against another electrode without KCl on the same tissues, yet there is no reason to suppose that these structures have a demarcation potential.

Eccles and Magladery (1937a) question the slow potential changes of the membrane described by Rosenblueth, Leese and Lambert (1933) because it appears unlikely to them that slow changes would record with diphasic leads. This argument would be valid if a slow change were uniform throughout the smooth-muscle cell. If the change in the cell were asymmetric—i.e., if one pole of the cell became enduringly electropositive with respect to the other pole— then a slow change would record readily with diphasic leads. Since the slow component 7 of the pilomotor electrograms is not an artifact (p. 267) it may be inferred that the suggestion of Rosenblueth, Davis and Rempel (1936), that smooth-muscle cells can exhibit asymmetric electrical changes, is corroborated by the data.

B. *The significance of the electric responses.* The appearance of an electric phenomenon in a tissue reveals the occurrence of an event and places it in time, but does not give any information as to the nature of the event other than that it has an electrical sign. On the basis of an assumed analogy the initial component *1* of the electrograms of the membrane and pilomotors was interpreted by Rosenblueth, Leese and Lambert (1933) as equivalent to the spike potential of striated muscle. It would then be indicative of a propagated wave similar to a nerve impulse. This interpretation was later rejected by Rosenblueth, Davis and Rempel (1936) because of several considerations which need not be repeated here.

Eccles and Magladery (1937a) adopted the view that, with the exception of a component prominent only on very weak stimulation (the N wave), all the components of the electrogram of the membrane were equivalent to the spike potential of striated muscle—i.e., that they denote propagated waves which obey the all-or-nothing principle and which are followed by a refractory state.

Eccles and Magladery interpreted the two deflections *1* and *2* as the two phases of a single diphasic wave. This supposed diphasicity was the main argument for inferring a wave of negativity which propagated beneath the electrodes from the orbit to the free border of the membrane. The inference is not supported by the present analysis, since there is not a diphasic wave, but two independent monophasic components of opposite sign, both in the membrane (fig. 10) and in the pilomotors (figs. 2 to 5).

A further argument used by Eccles and Magladery (1937a) in favor of the conducted nature of the electric phenomena was an occasional change in the latency of the peak of *1* when one lead was kept fixed at the free border of the membrane and the other was shifted to different positions on the external surface of the organ. As pointed out by Acheson (1938) these results may not be explained on the basis of conducted waves, for the positions of the movable

electrode were such that there were no underlying muscle elements in the majority of the observations.

Were there any components of the electrograms of the membrane and pilomotors analogous to the spike potential and the negative after-potential of striated muscle, it would be expected that veratrine should elicit changes of these components similar to those it evokes in the corresponding electric phenomena in the striated tissue. This expectation is not fulfilled by the data (pp. 270, 275).

Eccles and Magladery (1937a) presented records which they interpreted as indicative of a refractory condition of some elements during the development of the rhythmic waves of the membrane. In the experiments illustrated in figure 12, there is no evidence of a refractory state at any time during the waves of the rhythmic component. These observations confirm Rosenblueth and Acheson's (1937) conclusion that the membrane does not have a refractory state.

The complexity of any muscle is such that it is not surprising that the electrogram should include several components, even if none of these electric phenomena corresponds to a propagated disturbance of the type which exists in striated muscle. As suggested by Rosenblueth, Davis and Rempel (1936), some of the components may correspond to the excitatory processes: the release and diffusion of the chemical mediator and the changes it undergoes before it exerts its action on the contractile system. In addition the mechanical changes may also have an electric sign. Finally, the recovery processes may in turn lead to electrical changes.

Because of the rough parallelism between the slow final component of the membrane and the development of tension, Bacq and Monnier (1935) assumed that the change of polarization was the cause of contraction. As pointed out before (p. 274) the membrane is not a suitable organ for the study of the slow components of the electrogram.

In the pilomotors, where the late components are readily identified, both *6* and *7* vary directly with the mechanical responses (p. 269). Since *6* coincides with the period of development of the mechanical effect it is probable that it is the electrical sign of the chemical changes which correspond to this development. Component *7*, on the other hand, clearly follows the mechanical events. It is reasonable, therefore, to interpret it as the manifestation of recovery processes.

C. *The differences between the membrane and the pilomotors.* As stated in the introduction these two smooth muscles were selected for study because many of their physiological properties are similar. It is interesting, therefore, to note that the similarity does not extend to all the electric responses.

The initial components *1* and *2* of the electrograms have similar properties in the two muscles, when tested by repetitive stimulation at slow frequencies or after injections of veratrine and of 933F. The electrogram of the membrane may sometimes exhibit a succession of waves reminiscent of components *3, 4, 5* and *6* of the pilomotors (fig. 1A and B). With the data on hand it is not warranted, however, to affirm that this similarity is more than superficial.

It is probable that the membrane has a prolonged final component analogous to component 7 of the pilomotors (see p. 274).

A striking difference between the two electrograms is revealed by the rhythmic component, very readily obtained in the membrane (figs. 11 and 12) and invariably absent in the pilomotors. A further difference is indicated by the persistence of electric responses in the pilomotors after injections of ergotoxine (fig. 5), as contrasted with their absence in the membrane (p. 275).

These differences in the electric responses of two otherwise quite similar muscles support the view stated in the introduction that smooth muscles form a heterogeneous class. It is not valid, therefore, to generalize from observations on one sample to the class as a whole.

SUMMARY

The electric and mechanical responses of the nictitating membrane and the tail pilomotors were studied in cats. Injections of cocaine, veratrine, 933F or ergotoxine were used in order to separate out various components in the electrograms and to examine the relations between electric and mechanical events.

The pilomotor electrogram exhibits 7 components (fig. 1A) which may vary independently (figs. 2 to 8). Components 1 to 5 show no correlation with the mechanogram. Components 6 and 7 vary, as a rule, with contraction (p. 269). Component 6 coincides with the development of the mechanical response (figs. 6B and 9C); it is interpreted as correlated with the chemical changes associated with this development. Component 7 outlasts contraction (fig. 9); it is assumed to correspond to recovery processes. Both slow components 6 and 7 can be recorded from "diphasic" leads, thus proving that they correspond to an asymmetric change in each muscular cell.

The first 2 components of the membrane electrogram (fig. 10) are similar to those of the pilomotors—they are monophasic waves of opposite polarity. The membrane has at least one component never encountered in the pilomotors, the rhythmic component (fig. 11B). Components 1 and 2 may develop, in response to additional stimuli, during the course of the rhythmic component (fig. 12). These several components are therefore independent.

The discussion is concerned with the methods for the study of the two smooth muscles (p. 275), with the significance of their electrograms and the features in which they differ from that of striated muscle (p. 276), and with the heterogeneity of smooth muscles as a class (p. 277).

REFERENCES

ACHESON, G. H.   Anat. Rec. **71**: 297, 1938.
ACHESON, G. H. AND A. ROSENBLUETH.   This Journal **133**: 736, 1941.
BACQ, Z. M. AND A. M. MONNIER.   Arch. Internat. Physiol. **40**: 467, 1935.
ECCLES, J. C. AND J. W. MAGLADERY.   J. Physiol. **90**: 31, 1937a.
          Ibid. **90**: 68, 1937b.
GRAHAM, H. T.   J. Pharmacol. and Exper. Therap. **39**: 268, 1930.
LAMBERT, E. F. AND A. ROSENBLUETH.   This Journal **114**: 147, 1935.
MONNIER, A. M. AND Z. M. BACQ.   Arch. Internat. Physiol. **40**: 485, 1935.

ROSENBLUETH, A. Biological Symposia 3: 111, 1941.

ROSENBLUETH, A. AND G. H. ACHESON. This Journal 120: 514, 1937.

ROSENBLUETH, A. AND W. B. CANNON. Ibid. 116: 414, 1936.

ROSENBLUETH, A., H. DAVIS AND B. REMPEL. Ibid. 116: 387, 1936.

ROSENBLUETH, A. AND E. C. DEL POZO. Ibid. 136: 699, 1942.

ROSENBLUETH, A., C. LEESE AND E. LAMBERT. Ibid. 103: 659, 1933.

ROSENBLUETH, A. AND D. McK. RIOCH. Ibid. 103: 681, 1933.

ROSENBLUETH, A., J. H. WILLS AND H. HOAGLAND. Ibid. 133: 724, 1941.

STUDIES ON MECHANISMS INVOLVED IN SHOCK[1]

E. MYLON, M. C. WINTERNITZ, R. KATZENSTEIN AND G. J. DE SÜTÖ-NAGY

From the Laboratory of Pathology, Yale University School of Medicine

The introduction into the circulation of thromboplastic substance, as is well known, results in intravascular clot formation. This may be extensive and may be followed promptly by death. Such an outcome has been explained quite naturally as the consequence of mechanical circulatory block. On the other hand, the clot varies considerably in quantity depending upon speed of injection and various factors involved in the phenomenon of coagulation (1). Indeed the clot may be insignificant in amount and so located, as for instance in a radicle of the portal vein, that it would be entirely incompatible with the mechanical explanation of the acutely fatal outcome of the experiment.

These observations led to more detailed study of the circulatory changes subsequent to the intravenous injection of thromboplastic substance. It was found that the primary stage of excitement so manifest with the unanesthetized dog is lacking when the animal is under the influence of nembutal. Then marked vasodepression occurs promptly. The blood pressure falls to 20 mm. Hg or less within $\frac{1}{2}$ minute and, depending largely on the amount and potency of the thromboplastic substance, returns to normal only after a varying period of a few minutes to a half-hour or more.

The rôle of blood coagulation in this vasodepressor phenomenon is elucidated by heparinization preliminary to the injection of thromboplastic substance. No fall in blood pressure occurs; furthermore, preliminary section of the cervical or thoracic spinal cord does not interfere with the formation of clot after the injection of thromboplastic substance but the characteristic rapid and sustained fall in blood pressure is lacking (2).

These observations, implicating clot formation and its action through the central nervous system in the hemodynamic changes effected by tissue extracts, led to further study involving other contrasting methods of causing vasodepression. These included the use of histamine, well known to cause prompt dilatation of the peripheral vascular bed (3, 4) and also the method of release of an arterial occluding tourniquet recognized to be followed by a gradual but persistent fall of blood pressure to "shock" levels (5, 6). At the same time shifts in fluid, protein and fibrinogen content of the blood were determined. The experiments were varied to include other factors that might bear upon mechanisms involved such as electrolyte balance and the influence of the nervous system as affected by section of the spinal cord and by anesthesia.

After a brief statement concerning materials and methods, a combination of procedures involving agent and animal in general accord with the following outline will be presented.

Changes in blood pressure, hematocrit, and plasma protein effected by *1*, the intravenous injection of thromboplastie substance; *2*, the intravenous injection of histamine; *3*, the release of an arterial oecluding tourniquet.

All (*1,2,3*) in *a*, anesthetized animals; *b*, anesthetized animals with seetion of spinal eord; *e*, anesthetized animals with reduced tissue potassium.; *d*, unanesthetized animals (group *3* not ineluded).

MATERIALS AND METHODS. Dogs were the animals of choiee; no particular eriterion in their seleetion exeept that of good general health was exereised. Nembutal (pento-barbital sodium) in 30 mgm. per kilo amounts was used for anesthesia and when an experiment continued for four hours or more a second intravenous injeetion of one-third the original quantity was found desirable. The saline extraet of the dried residue after aeetone-ether extraetion of minced pig testiele was the thromboplastic substanee used; the histamine was the dihydroehloride (Eastman). For interference with the eirculation of the back legs thick-walled rubber tubing was applied just below the great trochanter of the femur on the outer side and as high as possiblc on the median side of each leg. The tourniquet was tightened to obstruet arterial circulation completely and left in this state for five hours. Low potassium diet was tolerated well by all but an occasional animal. Low potassium diet as indicated in the following formulae was supplemented daily with the B complex: Thiaminc 100 μg., Riboflavine 25 μg., Pyridoxine 60 μg and nicotinic aeid 25 mgm. per kilo of the animal's weight.

Cascin, 9000 grams	Magnesium sulphate, 172 grams
Cane sugar, 24,000 grams	Di sodium phosphate, 288 grams
Crisco, 7000 grams	Mono calcium phosphate, 722 grams
Cod liver oil, 800 grams	Calcium lactate, 172 grams
Sodium chloride, 172 grams[2]	Ferric ammonium citrate 72 grams

The animals lost from 20 to 25 per eent of their original weight but remained in general good condition during the 3 to 4 weeks required for adequate reduetion of tissue potassium. The blood pressure reeords with a mercury manometer were made from the femoral artery except when the experiments involved the back legs. Then they were made from the earotid. The hematocrit determinations were secured with 15 cc. of blood plaeed, as soon as drawn, in a graduated tube containing 30 mgm. of sodium oxalate. The tube was promptly rotated at 2000 r.p.m. for 25 minutes prior to reading. Then the plasma was removed for protein including fibrinogen eontent values and other determinations. One eubie centimeter of this fluid was diluted with distilled water to 100 ec. of whieh 20 ec. was used for the determination of nitrogen by the Kjeldahl method and another sample was required to ascertain fibrinogen eontent with the aid of protamine (9).

Changes in blood pressure, hematoerit and plasma protein effeeted by the intravenous injection of thromboplastic substance in animals (a) under nembutal anesthesia. The record that follows (dog 1) is typieal. The blood pressure fell from

---

[2] Ten grams of the diet contained less than 1.5 mgm. K (7). The K content of the skeletal and heart muscle of dogs fed this diet for a period of over 3 weeks is reduced as much as 25 per cent (own unpublished results). These figures are in accord with results by Darrow and Miller (8).

160 to 42 mm. Hg within ½ minute and the persistence of this low level for 6 minutes was associated with clot formation and a prompt shortening followed within 1 minute by lengthening of the coagulation time. The blood pressure almost reached the pre-injection level within ¼ of an hour and the coagulation time was prolonged for a much longer period.[3]

A second animal in this series of seven follows. Here blood pressure, hematocrit and plasma protein changes are correlated. The loss of fluid from the blood
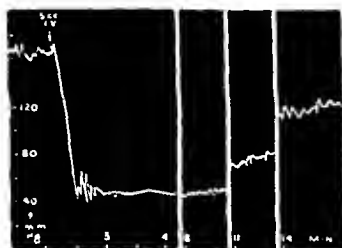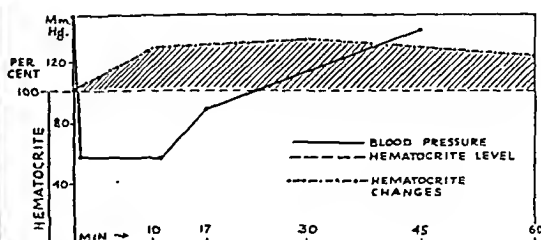
Fig. 1   Fig. 2

Fig. 1. Dog 1. The effect of intravenous injection of testicle extract upon the blood pressure of a normal anesthetized dog (condensed curve).

Fig. 2. Dog 2. Hematocrit and blood pressure changes after intravenous injections of testicle extract in a normal anesthetized dog.

TABLE 1

The effect of the intravenous injection of thromboplastic substance on the anesthetized (Nembutal) dog 2

	HEMATOCRIT	PLASMA PROTEIN	FIBRINOGEN
	per cent	*per cent*	*per cent*
Before injection..............................	42.0	5.86	0.333
10' after injection.............................	53.0	5.75	0.100
30' after injection.............................	55.6	4.86	0.103
60' after injection.............................	51.0	5.51	0.149

Variation in per cent of the original values (corrected for the change in hematocrit). They represent the loss of plasma protein per unit of whole blood

10'...		−28.0	−96.0
30'...		−49.0	−100.0
60'...		−27.3	−76.6

practically reached its peak in 10 minutes after injection and with minor variation remained fixed for more than an hour even though the blood pressure had reached its pre-injection level at a considerably earlier period. The plasma protein continued to fall, only attaining its maximal loss after a half-hour and not returning

---

[3] Autopsy notes, dog 1. "There are fresh, stringy dark blue thrombi in some of the branches of the portal vein but they do not occlude the lumen of the vessel. There is some thrombosed, light gray or gray-red material, not very extensive, twined around the chordae of the tricuspid valve.—Lungs and Liver: Without hemorrhage or thrombi."

to normal an hour after the experiment was begun. The fibrinogen loss from the plasma reached its height after 10 minutes and only a slight return was observed an hour later. These facts are illustrated in table 1 and figure 2.

It should be recorded that the fibrinogen determinations were done in parallel series using the methods of Cullen and Van Slyke (10) and Wu and Ling (11) for fibrin, and protamine for fibrinogen (9). The fibrin yield at the 10 minute and 30 minute readings was negligible, confirming older observations in this particular and leading to the conclusion that injection of tissue extract results in defibrinogenation of the blood. The protamine method showed, however, that though the fibrinogen is decreased it still is present in 0.1 per cent amounts or more; in other words, quantitatively adequate for clot formation. The absence of fibrin, therefore, is not dependent on inadequate fibrinogen, but to the practically complete disappearance of prothrombin (12) as determined by Quick's method (13).

Briefly stated, injection of thromboplastic substance into the anesthetized dog is followed by marked changes in blood coagulation time, pressure, hematocrit and plasma protein content. These changes have the same general trend but

TABLE 2

The effect of the intravenous injection of thromboplastic substance on the heparinized dog 3

	HEMATOCRIT	PLASMA PROTEIN
	per cent	*per cent*
Before injection of thromboplastic substance...........	31.0	6.1
10' after injection of thromboplastic substance.........	28.7	6.1
27' after injection of thromboplastic substance.........	27.0	6.0
50' after injection of thromboplastic substance.........	31.0	6.3
90' after injection of thromboplastic substance.........	30.0	5.8

their extent, duration, and return to pre-injection levels are in no way coincident. The fibrinogen loss is especially marked with the intravenous use of thromboplastic substance but a sufficient quantity remains in the plasma to effect fibrin formation and clotting, were it not for change in prothrombin.

The rôle of clot formation in the changes of the blood pressure and red cell volume after intravenous injection of thromboplastic substance is readily demonstrated by preliminary heparinization or by reinjection when the clotting time is still greatly lengthened. Examples follow:

Dog 3 received 8 ampules (400 mgm. of the sodium salt of heparin) of Liquaemin intravenously over a period of 12 minutes. Eight minutes later it received 2 cc. of testicle extract by the same route. The clotting time three minutes after heparinization was more than twenty minutes and continued unchanged at fifty minutes.

The slight transient drop in blood pressure is associated with peripheral vasodilator substances in the tissue extract and is not pertinent to the present study. Likewise, there is no significant shift in the fluid or blood protein (table 2, figure 3).

Careful examination of all the larger vessels grossly as well as detailed histological search failed to reveal thrombus formation in this animal or in any of the others of this series of eight.

Very similar results were secured when animals were reinjected with thromboplastic substance during the period of extended coagulation time soon after previous utilization of such an agent. This is illustrated in the blood pressure curve that follows (dog 4).

It will be recalled (1) that such reinjection is unassociated with symptoms irrespective of speed of introduction or amount of material used and that evidence of clot formation after many weeks of this treatment usually is entirely lacking.

These experiments stress the importance of clot formation in the changes in blood pressure, hematocrit and plasma protein content that follow the injection of thromboplastic substance into the anesthetized dog.

b. *Anesthetized animals with transection of the spinal cord.* Further information of the mechanism involved when clot follows the intravenous injection of thromboplastic substance is available from a series of experiments involving section of the cervical or thoracic spinal cord. Dog 5 is representative of a group of

Fig. 3   Fig. 4
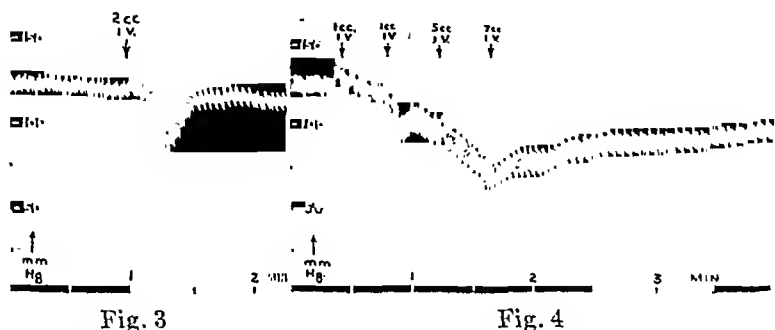
Fig. 3. Dog 3. The effect of intravenous injection of testicle extract upon the blood pressure of a heparinized anesthetized dog. Note the slight and transient drop in contrast to the non-heparinized animal.

Fig. 4. Dog 4. Small doses of testicle extract injected slowly into an anesthetized dog cause only a slight drop in pressure but lead to a negative clotting period during which the injection of even large doses is not followed by the usual fall in pressure.

four animals in which detailed analyses of the blood were made. Many others were included in the study of the effect of spinal cord section on the blood pressure.

As is illustrated in figure 5, the drop in pressure after the injection of thromboplastic substance is not great and almost immediately begins to swing back toward normal. It has been pointed out that this is due to substances in the extract acting directly on the peripheral vessels. The figure shows further that there is a distinct tendency to hemodilution expressed to a variable degree in different animals. The plasma protein content either is not or only slightly changed. This is illustrated in table 3.

Attention should be directed to the fibrinogen values included in table 3. They show only slight decrease even after a second large injection of thromboplastic substance, a fact which contrasts with the effect of such injection in an animal with intact spinal cord. This is the more noteworthy as there was less change in the prothrombin time.

These experiments indicate that the central nervous system mediates the blood

pressure fall and also the changes in red cell volume and plasma protein that follows clot formation after the intravenous injection of thromboplastic substance into the anesthetized dog. Even when such clot is demonstrated in large amount
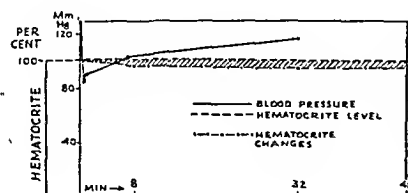

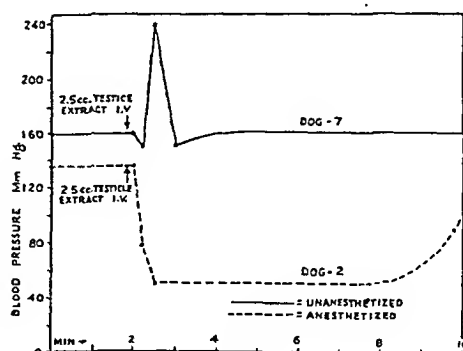Fig. 5   Fig. 6

Fig. 5. Dog 5. Intravenous injection of testicle extract into an anesthetized dog after transection of spinal cord. Note the slight and transient drop in pressure and the lack of significant changes in the hematocrit.

Fig. 6. Dogs 2 and 7. Comparison of blood pressure reaction after the injection of testicle extract in unanesthetized and anesthetized animals. Note the short, sharp rise in the unanesthetized animal (no. 7) in contrast to the sharp and protracted drop in pressure in the anesthetized animal (no. 2).

TABLE 3

The effect of the intravenous injection of thromboplastic substance on the anesthetized dog after transection of the spinal cord

	HEMATOCRIT	PLASMA PROTEIN	FIBRINOGEN
	per cent	*per cent*	*per cent*
Before transection of cord.........................	51.8	5.99	0.206
After transection of cord.........................	50.0	5.94	0.212
1. *Injection of testicle extract 2.5 cc.*			
9' after injection of testicle extract...........	48.7	5.56	0.213
30' after injection of testicle extract..........	47.3	5.67	0.239
2. *Injection of testicle extract 20 cc. (33' after the 1st)*			
30' after 2nd injection......................	48.6	5.38	0.179
120' after after 2nd injection..................	49.7	5.22	0.179

Variation in per cent of the original values (corrected for the change in hematocrit)

9' after 1st injection...........................	−4.0*
30' after 1st injection..........................	+1.0
30' after 2nd injection..........................	−6.5
120' after 2nd injection.........................	−12.0

* These figures represent the loss of plasma protein per unit of *whole blood*.

no essential changes occur in blood pressure or in the fluid or protein of the blood when the spinal cord has been severed. Indeed there is a tendency to hemodilution and occasionally an actual increase in the plasma protein.

c. *Anesthetized animals with reduced tissue potassium.* Alteration in electrolyte

balance through diet suggested itself as another approach to the study of the rôle of the neural mechanism in the hemodynamic changes that follow the injection of thromboplastic substance in the anesthetized animal. The potassium-free diet was chosen on the basis of the well-known relation of this ion to cell-membrane permeability and autonomic nervous activity. Dog 6 is representative of this group. Following the injection of thromboplastic substance the same type and extent of vasodepression occurred as in the normal anesthetized dog 2 (see fig. 2). On the other hand there was no hemoconcentration and no change in plasma protein half an hour after the injection of 4 cc. of testicle extract. A second injection of a much larger amount of extract—20 cc.—caused a like fall in blood

TABLE 4

The effect of the intravenous injection of thromboplastic substance on the anesthetized dog with reduced tissue potassium. Dog 6. Low potassium diet for 23 days

	HEMATOCRIT	PLASMA PROTEIN	FIBRINOGEN
	per cent	*per cent*	*per cent*
Before injection, 4 cc. testicle extract.............	40.0	4.47	0.514
11' after injection...............................	42.0	4.59	0.270
28' after injection...............................	41.0	4.38	0.285
2nd injection (31' after the 1st injection 20 cc. testicle extract)			
10' after 2nd injection.........................	40.4	4.04	0.049
60' after 2nd injection.........................	45.6	4.42	0.236
225'...	49.2	4.47	0.203
285'...	43.4	4.13	0.224

Variation in per cent of the original values (corrected for the change in hematocrit)		
11'..		−2.0	
28'..		−4.5	
2nd injection			
10' after 2nd injection......................		−10.0	
60'..		−15.0	
225'...		−22.0	
285'...		−15.0	

pressure which was more protracted and was associated with a slight transient hemoconcentration and loss of plasma protein. This is illustrated in table 4.

It should be pointed out that the improvement in the animal's condition was temporary and death occurred $5\frac{1}{4}$ hours after the first injection of extract. Many experiments with normal dogs that invariably recover after a second large injection of thromboplastic substance indicate that protracted low potassium diet renders an animal less resistant. As will be seen, none of the latter type of animal survived the procedures to be described in the course of this presentation. While they do not seem to be as sensitive to experimental procedures as adrenalectomized animals, they also are not as resistant as the normal dog, in spite of the fact that the hematocrit and plasma protein changes are much less marked. This is supporting evidence that these alterations in the blood are not primarily causative in the death of the animal.

The interpretation of the findings after injection of thromboplastic substance in the animal whose tissue potassium has been depleted is not easy. Potassium deficiency in itself is associated with some change in tissue and blood fluid with resultant influence on membrane, including capillary permeability. This well may be a cause for the greater stability of the capillary wall in the dog, largely depleted of potassium. On the other hand, the influence on the neural mechanism also must be borne in mind.

d. *The unanesthetized animal.* Considerable experience with thromboplastic substance in the unanesthetized dog, as has been said, indicated that its intravenous injection was accompanied by excitement and central nervous system stimulation rather than the depression described for the anesthetized animals. For this reason more detailed studies of blood pressure and blood constituents now to be recorded, were made. The reactions to injection displayed by dog 7 are characteristic for this series. Initial stimulation associated with injection of testicle extract is manifested by an immediate acute elevation of blood pressure

TABLE 5

The effect of the intravenous injection of thromboplastic substance in the unanesthetized dog

	HEMATOCRIT	PLASMA PROTEIN
	per cent	*per cent*
Before injection of 2.5 cc. thromboplastic substance	38.2	5.49
10′ after injection of 2.5 cc. thromboplastic substance ...	38.8	5.09
Second injection of 20 cc.		
20′ after 1st injection of thromboplastic substance		
13′ after 2nd injection of thromboplastic substance....	38.9	5.29
73′ after 2nd injection of thromboplastic substance.....	37.9	5.14
135′ after 2nd injection of thromboplastic substance....	36.4	4.83

lasting only a minute or so and followed by a return to the pre-injection level, where it remained with only minor variations. This contrasts sharply with the reaction of the nembutalized animal, as is illustrated in figure 6.

An occasional unanesthetized dog reacts so violently to injection of thromboplastic substance that blood pressure readings become impossible (more than 300 mm. Hg). A second later injection at the usual speed of a large quantity of extract (20 cc.) may be followed by a drop in pressure of 50 or more mm. Hg with a return in a few minutes. Clot formation occurs regularly and may involve the cannula to interfere with manometric recording.

In dog 7, as has been the experience with all of the animals in this group, both the hematocrit and the plasma protein determinations have shown no significant changes. This is illustrated in table 5.

SUMMARY. Anesthesia with nembutal allows the full expression of vasodilatation, change in red cell volume and plasma protein content clot formation which thromboplastic substance is capable of eliciting. Section of the spinal cord eliminates these reactions after clot formation while the normal dog actually reverses the effects with an increased blood pressure response, occasionally hemodilution, and even increased plasma protein.

While the general trend of change in blood pressure and blood constituents is the same, sufficient quantitative and temporal variation occurs to allow the conclusion that they are in part at least determined by independent influences.

It should be pointed out at this time that contraction of the spleen with resultant pouring into the circulation of its concentrated red blood cell content may participate up to 15 per cent in increased red blood cell volume (14). Associated loss of plasma protein cannot be explained on this basis and requires further consideration. Increased permeability of the capillary wall to fluid and protein is the mechanism that suggests itself. That splenic contraction cannot be the decisive factor in the hemoconcentration recorded for the experiments in this series is shown by comparison of the findings after injection of thromboplastic substance in anesthetized animals with and without depletion of tissue potassium. Dog 2, table 1, shows a 32 per cent hemoconcentration and 17 per cent loss in plasma protein during the first 30 minutes contrasting with dog 6, table 4, with negligible changes in these values during the same period. Depletion of tissue potassium as a result of restricted intake of this electrolyte definitely reduces the changes in hematocrit and plasma protein content even though it does not exert any influence on the depressor action of the tissue extract. It should be noted further that animals with depleted tissue potassium are less resistant to experimental procedures, but this is not as marked as after adrenalectomy.

Fibrinogen change requires special consideration when thromboplastic substance is used intravenously. However, its disappearance exceeds what could be anticipated from the size of the resultant clot. Some other physical change in this protein, its sticky state in particular, may be responsible in part for the apparent reduction.

2. Changes in blood pressure, hematocrit and plasma protein effected by the intravenous injection of histamine. A. In the anesthetized animal. Histamine was selected to contrast with thromboplastic substance for it acts, as is well known, primarily on the peripheral vascular bed with a resultant drop in the pressure of the blood and increased capillary permeability. On the other hand, the anesthetized animal differs markedly in its response to histamine from the unanesthetized dog and still further variation is encountered in the anesthetized animal with transected spinal cord, as will be shown.

Figure 7 is characteristic of the blood pressure and hematocrit changes that occur after the intravenous injection of 5 mgm. of histamine in the normal anesthetized (nembutal) dog 8 (0.9 mgm. per kgm.).

It will be seen that the marked drop in blood pressure begins to be corrected promptly and that the hematocrite change is still outspoken after the pressure has approached its former height.

In the second animal, dog 9, with approximately the same effect on the blood pressure, the hematocrit value rose considerably higher and this was sustained for a protracted period after the blood pressure had returned to normal. The plasma protein and fibrinogen loss, like that of the water,[4] was extensive. This is illustrated in table 6.

---

[4] Changes of red cell volume of this magnitude cannot be ascribed to the consequences of splenic contraction (14).

B. *Anesthetized animals with transection of the spinal cord.* Only two animals were included in this group. Their reactions to the injection of histamine were similar in every detail. The fall in blood pressure with its gradual but prompt rise towards normal corresponded to that of the normal dog. In contrast to this latter type of animal, the pressure change was not accompanied by a marked hemoconcentration; there was but a negligible loss of plasma protein and fibrino-
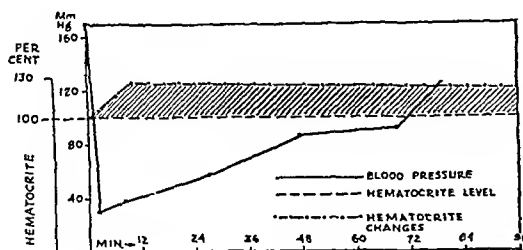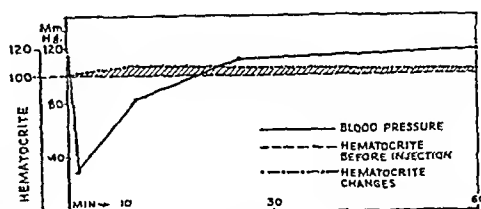

Fig. 7   Fig. 8

Fig. 7. Dog 8. Hematocrit and blood pressure changes after intravenous injection of histamine in a normal anesthetized animal.

Fig. 8. Dog 10. Intravenous injection of histamin into an anesthetized dog after transection of the spinal cord. Note the absence of significant changes in hematocrit in contrast to dog 8 in figure 7.

TABLE 6

The effect of the intravenous injection of histamine on the anesthetized dog 9

	HEMATOCRIT	PLASMA PROTEIN	FIBRINOGEN
	per cent	*per cent*	*per cent*
Before injection of 5 mgm. of histamine (0.4 mgm. per kgm.)	45.2	5.73	0.336
10′ after injection	61.5	5.57	0.219
60′ after injection	71.8	5.07	0.226
120′ after injection	80.9	5.60	0.156
180′ after injection	80.3	5.09	0.214
270′ after injection	76.0	4.20	0.236
Variation in per cent of the original value (corrected for the change in hematocrit)			
10′ after injection		−41.0	−73.0
60′ after injection		−72.0	−92.0
120′ after injection		−84.0	−134.0
180′ after injection		−89.0	−114.0
270′ after injection		−97.0	−100.0

gen. (See table 7 and fig. 8.) This, it may be recalled, is in accord with the results obtained with thromboplastic substance. The fall in blood pressure with the associated hemoconcentration and loss in plasma protein and fibrinogen manifested by the normal animal gives way to a fall only in pressure, a tendency to hemodilution, and occasionally actual increase in plasma protein when the spinal cord is severed.

C. *Anesthetized animals with reduced tissue potassium.* Three dogs are included in the group subjected to a preliminary period of about 30 days on a low potas-

TABLE 7

The effect of the intravenous injection of histamine in the anesthetized dog after transection of the spinal cord. Dog 10. Spinal cord cut. Nembutal anesthesia—5 mgm. histamine

	HEMATOCRIT	PLASMA PROTEIN	FIBRINOGEN
	per cent	*per cent*	*per cent*
Before transection of the cord.....................	57.7	5.51	0.306
After transection of the cord.....................	48.6	5.8	0.271
10′ after injection of 5 mgm. of histamine (0.3 mgm. per kgm.)..	52.0	5.47·	0.259
25′ after injection of 5 mgm. of histamine.........	51.1	5.64	0.238
60′ after injection...............................	50.0	5.38	0.250
130′ after injection..............................	48.9	5.76	0.249
260′ after injection..............................	51.8	5.34	0.298

Variation in per cent of the original values (corrected for the change in hematocrit)

10′ after injection...............................		+1.0	+13.0
25′ after injection...............................		+7.0	−17.0
60′ after injection...............................		+4.0	−10.0
130′ after injection..............................		+13.0	−8.0
260′ after injection..............................		±0	+4.0

TABLE 8

The effect of intravenous injection of histamine on the anesthetized dog with reduced tissue potassium. Dog 11 for 30 days

	HEMATOCRIT	PLASMA PROTEIN	FIBRINOGEN
	per cent	*per cent*	*per cent*
Before injection of 5 mgm. of histamine (0.4 mgm. per kgm.)..	44.0	5.84	0.275
10′ ′after injection...............................	53.9	5.90	
60′ after injection...............................	52.3	5.56	0.289
120′ after injection..............................	52.4	5.18	0.271
240′ after injection..............................	51.9	5.58	0.268

Variation in per cent of the original values (corrected for the change in hematocrit)

10′..		−22.0	
60′..		−23.0	
120′...		−29.0	
240′...		−23.0	

sium diet. Under nembutal anesthesia they reacted similarly to the injection of 5 mgm. of histamine. There was a prompt and protracted fall in blood pressure with the same trend to the pre-injection level and the same recovery period

manifested by the normal dog. The extent of the hemoconcentration, however, as well as the loss in plasma protein and fibrinogen was far less than in the normal animal. This is illustrated in table 8.

D. *Unanesthetized animals.* The symptoms following injection of 5 mgm. of histamine in the unanesthetized dog are much less definite than in the animal under nembutal anesthesia. Indeed, 3 or 4 times the amount per kilogram are required to secure anything like the same protracted vasodepressor effect. This is also true for the changes in hematocrite and plasma proteins as is illustrated in table 9.

SUMMARY. Nembutal anesthesia results in greater susceptibility than the un-

TABLE 9

The effect of intravenous injection of histamine on the unanesthetized dog 12

	HEMATOCRIT	PLASMA PROTEIN
	per cent	*per cent*
Before injection of 20 mgm. histamine (1.3 mgm. per kgm.).	37.8	5.37
13' after injection of histamine.	45.4	5.23
43' after injection of histamine.	42.8	5.12
85' after injection of histamine.	39.4	5.12
155' after injection of histamine.	43.1	5.37
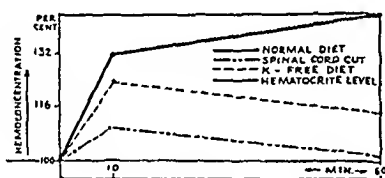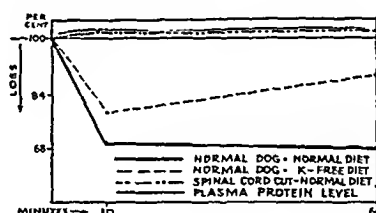
Fig. 9.   Fig. 10.

Fig. 9. Average hematrocrit changes following intravenous histamin injection into normal dogs, spinal dogs, and dogs with reduced tissue potassium.

Fig. 10. Average plasma protein changes after intravenous histamin injections into normal dogs, spinal dogs, and dogs with reduced tissue potassium.

anesthetized animal displays to changes in blood pressure, fluid, and plasma protein content after intravenous injection of histamine.

After prolonged feeding with a diet low in potassium, the anesthetized dog does not differ from the animal on a regular diet in his blood pressure reaction to the injection of histamine. Hemoconcentration and loss in plasma protein however are definitely reduced.

After section of the spinal cord the blood pressure fall and return conform to the pattern in the normal animal but hemoconcentration and loss in plasma protein do not occur. The tendency, though slight, is for hemodilution and for actual increase in protein. This is illustrated in figures 9 and 10.

3. *Changes in blood pressure, hematocrit and plasma protein effected by the release of an arterial-occluding tourniquet.* The unanesthetized dog was not utilized in

this series of experiments and no data are available for comparison with anesthetized animals included in the following presentation.

The release of the arterial-occluding tourniquet was followed invariably by a gradual but continued drop in blood pressure to shock levels of 60 to 70 mm. Hg.
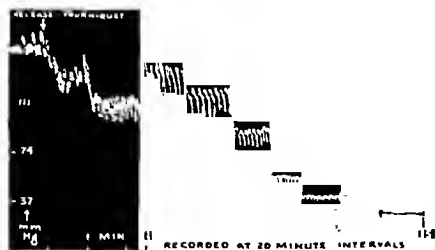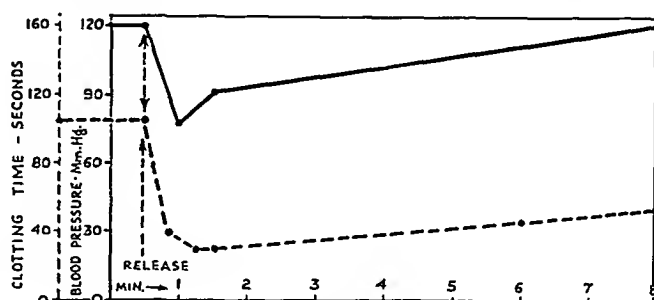
Fig. 11   Fig. 12

Fig. 11. Dog 13. Blood pressure fall following the release of tourniquets oecluding the eirculation of the hind legs of anesthetized dog 13.

Fig. 12. Dog 14. Changes in elotting time of the blood associated with the immediate and transient blood pressure fall after release of hind leg tourniquets. Note the shortening of the elotting time simultaneous with the pressure fall.

TABLE 10

The effeet of release of tourniquets occluding the circulation of the hind legs of the anesthetized dog 13. Ligation for 5½ hours. Death 2 hours after release

	HEMATOCRIT	PLASMA PROTEIN	FIBRINOGEN
	per cent	*per cent*	*per cent*
Before release of tourniquets.....................	42.0	5.66	0.541
10′ after release.................................	49.4	5.67	0.473
40′ after release.................................	55.4	5.83	0.572
60′ after release.................................	55.0	5.55	0.483
90′ after release.................................	54.0	5.06	0.600
120′ after release.................................	55.0	4.97	0.621

Variation in per cent of the original values corrected for the change in hematocrit

10′ after release of tourniquets..................	−17.0	−29.0
40′ after release of tourniquets..................	−26.0	−25.0
60′ after release of tourniquets..................	−39.0	−41.0
120′ after release of tourniquets..................	−43.0	−16.0

As a rule it continued for a period at this height but gradually decreased later in the experiment.

a. *Anesthetized animals.* Of the large group subjected to this procedure dog 13 is typical.

The blood pressure tracing (fig. 11) shows the downward trend after release of the tourniquet until it indicated only 30 mm. Hg shortly before the death of the animal after 115 minutes. The short, sharp fall followed by temporary rise in

pressure immediately after the re-establishment of the circulation requires comment. It was not a constant finding and both the fall and rise were variable in amount. A more extensive change of this type occurred in dog 14 and in this experiment as in several others the blood coagulation was studied. The figures are of interest and are placed in relation to the blood pressure curve as closely as could be ascertained. This is illustrated in figure 12.

Careful search for intravascular clots after the death of the animal was only rarely positive and the coagula were never large. Still it seems possible that the release of the circulation, which was associated with a shortened coagulation time, did cause small clots occasionally and that this is one of the causes for the primary drop in blood pressure. Further support for this viewpoint is forthcoming from the fact that this early immediate change in blood pressure does not occur in a heparinized animal. Other observers have noted clotting in the tip of the can-

TABLE 11

The effect of release of tourniquets occluding the circulation of the hind legs after transection of the spinal cord in the anesthetized dog 15

	HEMATOCRIT	PLASMA PROTEIN
	per cent	*per cent*
Before ligation..	40.4	4.98
Before transection of the cord.........................	43.5	4.17
Before release of tourniquets.........................	38.8	4.41
10' after release.......................................	43.9	4.88
60' after release.......................................	40.8	4.48

Variation in per cent of the original values corrected for the change in hematocrit		
Before release of tourniquets...........................	.	+17.0
10' after release......................................		+17.0
60'...		+14.5

nula immediately after the re-establishment of circulation in similar circumstances. The outcome of the experiment, however, is not influenced by absence of this primary change in coagulation time and small clot formation.

To continue with dog 13 (fig. 11), analysis of table 10 shows that hemoconcentration reached its height at the end of 40 minutes when the blood pressure was 116 mm. Hg. The plasma protein continued to drop until the death of the animal and the fibrinogen which had decreased maximally during the first hour again rose and almost reached the starting value before the experiment terminated.

b. *Anesthetized animals with transection of the spinal cord.* Dog 15 is selected from a group of seven animals whose spinal cords were cut at the level of the 2nd thoracic vertebra 4 to 5 hours after the circulation of the legs had been shut off and just before the occluding tourniquet was removed. Aside from the fact that the blood pressure usually was slightly reduced even before the circulation of the legs was re-established, it then fell gradually as in the other two groups of this series. The hematocrit on the other hand remained remarkably constant. The

plasma protein occasionally showed a tendency to reverse the picture of the normal dog in the same circumstances.

c. *Anesthetized animals with reduced tissue potassium.* The blood pressure

TABLE 12

The effect of release of tourniquets occluding the circulation of the hind legs of the anesthetized dog 16 with reduced tissue potassium

	HEMATOCRIT	PLASMA PROTEIN
	per cent	per cent
Before applying tourniquets.............................	47.4	5.49
Before release...	51.0	5.25
10' after release......................................	58.0	5.25
30' after release......................................	58.0	5.28
70' after release......................................	58.0	4.99
140' after release.....................................	66.0*	5.51*

Variation in per cent of the original values corrected for the change in hematocrit	
Before release of tourniquet............................	−14.0
10' after release......................................	−14.0
30' after release......................................	−13.0
70' after release......................................	−19.0
140' after release.....................................	−24.5*

* Agonal.

TABLE 13

The effects produced before release of tourniquets occluding the circulation of the hind legs of the anesthetized dog

DOG NO.	HEMATOCRIT		PLASMA PROTEIN	
	Value	Change	Value	Change
	per cent	per cent	per cent	per cent
17—Normal..............................	45.5 47.3	+4.0	5.81 5.66	−6.5
15—Normal..............................	40.4 43.5	+7.8	4.98 4.17	−24.0
16—Reduced tissue potassium...............	47.4 50.8	+7.0	5.49 5.25	−11.4
18—Reduced tissue potassium...............	49.3 51.0	+3.5	6.50 4.81	−26

curve requires no comment inasmuch as it conforms generally to that of the normal dog. The hematocrit, plasma protein and fibrinogen changes from the time of release of the circulation of the leg are all slight except for the agonal figures which must be discounted. This is illustrated in table 12 compiled for

dog 16, an example of a group of nine animals subjected to this particular procedure.

It will be noted from tables 11 and 12 that there is a distinct change in the hematocrit between the period of application of the tourniquet to the leg and its release. With only one exception out of eight animals, hemoconcentration occurred. This varied from an increase of 3.5 per cent to 7.8 per cent. The plasma protein loss in the same period was even more marked from 6 per cent to 26 per cent.

These changes in hematocrit and plasma protein are particularly noteworthy as they occurred during the period when the blood pressure was stable and when absorption from the legs with occluded circulation must have been minimal.

SUMMARY. The blood pressure curves in the three groups of animals are quite comparable. After four to five hours the tight tourniquet itself causes some hemoconcentration and loss in plasma protein. Transection of the spinal cord tends to lower the blood pressure slightly and to decrease the survival period. Especially conspicuous is the reduction of hemoconcentration and loss of plasma protein in animals preliminarily fed on a low potassium diet and the tendency to hemodilution and even absolute increase in plasma protein when the spinal cord has been severed.

DISCUSSION. Anesthesia, spinal cord section and depletion of tissue potassium have all been shown to have similar alterative effects upon the well known changes in blood pressure, red cell volume and plasma protein induced by intravenous injection of thromboplastic substance or histamine as well as by the release of an arterial occluding tourniquet on the hind legs. That the central nervous system is implicated in the controlling mechanism is strongly suggestive. Further analysis follows:

In the unanesthetized dog the clot resulting from injection of thromboplastic substance causes only a short, sharp rise in blood pressure; under nembutal anesthesia an acute protracted drop in pressure occurs. Since the stimulating agent is the same and the only difference is the block created by the anesthetic, it may be assumed that the vasopressor as well as the vasodepressor centers are activated simultaneously in the unanesthetized animal. As the vasopressor influences are recognized to be stronger, the drop in pressure is not manifest. Under nembutal, interference with central regulation of the vasopressor system occurs and depressor changes prevail as a result of the removal of this balancing agency. The pathway through which the depressor effects are transmitted are not established further than that they are contained in the spinal cord. As a consequence when the cord is transected the depressor reactions are greatly reduced and changes in blood pressure do not occur.

If it can be assumed that elimination of the pressor effect is an expression of lack of sympathetic control then depressor dominance may be considered as an expression of parasympathetic overbalance. In this association it should be recalled that nembutal is one of the few barbiturates said not to involve the parasympathetic (15).

When such dominance of the parasympathetic system exists it is expressed

primarily by a fall in blood pressure. Hemoconcentration and loss of plasma protein also occur, and these, too, may be considered as expressions of parasympathetic over function. This is supported by the observation that when the parasympathetic influence is minimized by section of the spinal cord, all of these manifestations disappear including fall in pressure, loss of fluid and protein from the blood.

It may be argued that in all of these experiments involving thromboplastic substance in the variously prepared animals, the changes in blood pressure and capillary permeability for fluid and protein have the same general trend. Even with this agent, however, their extent, duration and recovery are in no way coincident. When the series of experiments with histamine or with release of an arterial occluding tourniquet is considered, it becomes quite evident that the fall in pressure and the permeability of the capillary wall are not necessarily expressions of a single influence. For example, with histamine vasodepression is equally marked in the anesthetized dog with intact or with transected spinal cord. In the former there is marked hemoconcentration and loss in plasma protein; in the latter there is actually hemodilution and occasionally even increase in plasma protein. Other equally outstanding differences between blood pressure depression and capillary permeability may be cited as, for example, the release of an arterial occluding tourniquet in the dog with intact or transected cord, etc.

These facts necessitate the conclusion that if anoxia of the capillary wall follows sustained vasodepression, it cannot be the dominant or exclusive cause of increased capillary permeability. As pointed out by previous investigators (16, 17), it would be difficult to exclude the nervous system as a controlling influence for permeability of the capillary wall; further, it would seem that as long as the sympathetic system is functionally adequate, hemoconcentration or loss in plasma protein would not occur. As soon as this system becomes impaired, whether through anesthesia or other influence, and dominance of the parasympathetic becomes possible, there follows increased permeation of fluid and protein through the capillary walls. Hemoconcentration therefore may be related to impaired sympathetic control without diminished parasympathetic action.

This seems to be a definite neural controlling mechanism. That a humoral factor may exert an influence also must be considered. The evidence that anoxia plays a rôle cannot be disregarded, even though, as stated above, it may only be of relative significance in the local effect on the capillary wall. Much attention has been directed to changes in the adrenal cortex associated with inadequate oxygen supply, whether this results from high altitude, insufficient pulmonary aeration, or reduced blood flow in the gland. Under-function of the adrenal cortex, accompanying inadequate oxygen supply, is well known to be associated with mobilization of tissue potassium. When it is recalled that this electrolyte is regarded as one of the mediators of the parasympathetic system, it is not astonishing that hemoconcentration follows under-functioning of the cortex of this gland. In support of this view is the fact that loss of fluid and of protein from the blood through the capillary wall is greatly reduced if the tissue potassium of the experimental animal has been depleted by dietary control with decrease of

potassium available for mobilization. Evidence in support of this statement is included in the foregoing presentation.

Briefly stated, it is suggested that any influence, and there are many, that allows the parasympathetic nervous system to dominate results in low blood pressure and increased permeation of fluid and protein through the capillary wall. Such influences include exhaustion or interruption of the balancing power of the sympathetic nervous system and direct stimulation of the parasympathetic system. The latter may result from central or peripheral influences and among these potassium must be included. Its mobilization through impaired adrenal cortical function is recognized and such impaired adrenal cortical function is now known to be associated with inadequate oxygen supply to the gland.

SUMMARY

Studies on mechanism are presented associated with fall in blood pressure, relative red cell volume and plasma protein content following intravenous injection of thromboplastic substance, histamine, and release of an arterial occluding tourniquet on the hind legs of dogs.

The conditions of the experiments in each of these three groups have been varied to include the influence of anesthesia, section of the spinal cord, and depletion of tissue potassium.

Clot formation, after intravenous injection of thromboplastic substance, is associated with a short, sharp rise in blood pressure without significant variation in red cell volume or plasma protein in the unanesthetized animal. With anesthesia there is a marked and sustained fall in blood pressure, an increase in red cell volume, and a loss of plasma protein. None of these manifestations occur in the absence of clot formation as is demonstrable by preliminary heparinization. They are eliminated also when clot follows the use of thromboplastic substance if the spinal cord has been sectioned.

Anesthesia reduces greatly the amount of histamine essential to produce the equivalent fall in blood pressure, increase in red cell volume, and loss of plasma protein required for the unanesthetized animal. Section of the spinal cord does not modify the vasodepressor effect; it does, however, eliminate the rise in red cell volume, and the loss in plasma protein.

The gradual fall in blood pressure that follows release of the arterial occluding tourniquet is not influenced by section of the spinal cord. On the other hand, the increase in red cell volume and loss in plasma protein is eliminated by this procedure.

Preliminary depletion of tissue potassium in all three groups is without influence on the blood pressure changes; it does reduce the rise in red cell volume and the loss of plasma protein.·

The rôle of the autonomic nervous system and the part played by electrolytes in mediating its relation to the changes in red cell volume and plasma protein is discussed.

REFERENCES

(1) WINTERNITZ, M. C., E. MYLON AND R. KATZENSTEIN. Yale J. Biol. and Med. 13: 595, 1941.

(2) MYLON, E., H. HOFF, R. KATZENSTEIN AND M. C. WINTERNITZ. Science **94**: 190, 1941.
(3) DALE, H. H. AND A. N. RICHARDS. J. Physiol. **52**: 110, 1918.
(4) DALE, H. H. AND P. LAIDLAW. J. Physiol. **52**: 355, 1919.
(5) ALLEN, F. M. Arch. Surg. **38**: 155, 1939.
(6) WILSON, H. AND N. W. ROOME. Arch. Surg. **32**: 334, 1936.
(7) THOMAS, R. M., E. MYLON AND M. C. WINTERNITZ. Yale J. Biol. and Med. **12**: 354, 1940.
(8) DARROW, D. C. AND H. C. MILLER. J. Clin. Investigation (in press).
(9) MYLON, E., M. C. WINTERNITZ AND G. J. DE SÜTÖ-NAGY. J. Biol. Chem. **143**: 21, 1942.
(10) CULLEN, G. E. AND D. D. VAN SLYKE. J. Biol. Chem. **41**: 587, 1920.
(11) WU, H. AND S. M. LING. Chinese J. Physiol. **1**: 161, 1927.
(12) MYLON, E., M. C. WINTERNITZ AND G. J. DE SÜTÖ-NAGY. To be published.
(13) QUICK, A. J. This Journal **114**: 282, 1936.
(14) BARCROFT, J. AND L. T. POOLE. J. Physiol. **64**: 23, 1927–28.
(15) SWANSON, E. E. J. Pharmacol. and Exper. Therap. **46**: 387, 1932.
(16) GELLHORN, E. Das Permeabilitaetsproblem. p. 330. J. Springer, Berlin 1929.
(17) HESSELMANN, J. Ztschr. f. Biol. **92**: 287, 1931–32.

THE RÔLE OF HORMONES IN THE INITIATION OF MATERNAL BEHAVIOR IN RATS

OSCAR RIDDLE, ERNEST L. LAHR AND ROBERT W. BATES

From the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y.

During the past several decades it has been strongly suspected that a hormonal factor or factors actually precipitates the maternal response. Stone (23) pioneered in an attempt to resolve the problem by breeding one of two rats in parabiosis; the results, however, were inconclusive. Ceni (2, 3, 4) brought this early epoch to an end by showing that the sex hormones (androgens, estrogens) are not the causative factor and that they actually tend to inhibit or suppress maternal care in dogs. Ceni also initiated broodiness in poultry by prolonged injections of massive doses of combined extracts or dried tissue from thyroids, adrenals, hypophyses and parathyroids. With a mixed pituitary extract, in which gonadotropin was considered the effective factor, Ehrhardt (6) reported a single case of what seemed to be maternal behavior in an adult nonparous monkey. Wiesner and Sheard (24) then showed that crude extracts of the hypophysis alone—all containing mixtures of the various pituitary hormones—were somewhat effective in initiating maternal behavior in virgin female rats. These authors thought the effective hormone was not prolactin, stating that "it seems exceedingly unlikely that the same factor is responsible for mammary secretion and maternal behavior."

After learning that broodiness in fowl seems to be induced only or chiefly by prolactin (19) the present study (preliminary reports, 20–22) was undertaken with the sole purpose of identifying the specific hormone or hormones (and somatic state) which, in coöperation with parts of the nervous system, conditions or determines the exhibition of parental or maternal care in the rat.

METHODS. The method used for testing for maternal behavior was a marked modification of that adopted by Wiesner and Sheard (24). The usual procedure was to start with intact rats aged 60 to 70 days and test their behavior daily for a preliminary period of 10 days. All rats used were from litters in which the sexes were separated at age 30 days, and presumably no rat had previous retrieving experience; the only possibility for such experience is that it retrieved a litter mate. Each rat was placed and remained in an individual cage (39 x 39 x 22 cm.) provided with nesting material (wood shavings), food and water. The rear of all cages (in racks) was against the wall of a moderately lighted room; all sides and the top of cages were of open-mesh wire (metal floor) which permitted good observation of the cage's contents. Operated animals were allowed 2–5 days for recovery before beginning their tests.

In making the test a small metal trap-door (upper-front of cage) was opened (some noise) after first removing the flat-type water bottle (so arranged as to

fasten the door) and a young rat 1 to 5 days old dropped or placed in the front part of the cage. Thereafter, during a period of 10 minutes the activities of the subject were observed and the items considered significant were recorded; four or five subjects were thus observed simultaneously. An effort was made to have the rat pups warm and active at the beginning of the tests, but it was not possible to attain more than moderate uniformity in this item. Though the rat usually found the proffered pup at once it was sometimes necessary to attract or even to lift the rat (subject) to the front of the cage where the observer could make sure that the subject became aware of the presence of the young. Nearly all tests were made during daylight hours but a few were made at night. All tests except those originally reported (20) were made in a room which was adequately heated in winter. Practically all of the behavior records were made by two of the authors. In several of the earlier tests the two recorders observed the same rats at the same time.

Observations made and recorded covered the following items: whether and at which moments the subject "nosed" the young, consumed food, licked and handled the young, bit the young, returned to the nest alone, cuddled the young where it lay, retrieved the young only or both retrieved and cared for the young in the nest, repaired the nest with or without the young being in it, or piled nest material over the young wherever it happened to lie. In the present summarization of results, however, other items are disregarded and only those subjects that retrieved young and cared for them in the nest on three or more successive days are recorded as having exhibited full parental behavior. In relatively few cases was this behavior observed on one or two days with failure on a third day. Rats which retrieved regularly, but rarely or never cuddled the retrieved young, were credited with one-half the full parental response.

All animals were tested with rat pups as described above for a period of at least 10 successive days prior to their acceptance for tests under injection. Only in the first series of tests (20) were any of the rats given any sort of "priming injection"; likewise the practice of "concaveation" (leaving the young overnight or longer with the test animal), largely used by Wiesner and Sheard (24), was abandoned as an undesirable complication. Indeed, in any thermophilic animal (e.g., the mice of Leblond, 12), and particularly in any rodent whose heat production has been decreased by hypophysectomy or thyroidectomy, this emphatic use of visual and contact stimuli seems capable (through other "satisfactions") of rendering it impossible to study the rôle of hormones in maternal behavior. Some rats of all categories studied by us were found to exhibit either a partial or a "full" maternal response ("normal reactors") during the 10 days of preliminary testing. These "normal reactors" were eliminated from all subsequent tests except those in which a stoppage of the parental response was the goal of the test; and for the latter purpose only rats (normal reactors or those made maternal by hormones) giving the full response were accepted.

All subjects which remained distinctly negative throughout their 10-day preliminary test were injected daily during the next 10 days with one or another hormone and the tests and observations continued precisely as before. Animals which did not become maternal (i.e., did not give "full" positive responses on

three successive days) before the end of the series of 10 injections were tested daily thereafter and responses occurring within the following 3 days were arbitrarily considered as due to the substance administered. Indeed daily testing was continued on all animals which remained negative, and after a suitable interval (10–15 days) they were given a second, or even a third, series of injections with another substance or hormone. The daily injection was given subcutaneously and usually followed the period of test and observation by a few hours.

Four further items concerning the response are notable. *a*. The licking of pups—particularly their genitals—as soon as the pup was found, and before carrying it to the nest, seemed to be done more assiduously by rats at or near the initiation of their maternal response; a few days later the proffered pups were more likely to be carried directly to the nest where licking occurred at intervals. *b*. Many or most rats that became maternal thereupon assumed a more aggressive attitude to the hand of the observer when the retrieved young was removed from the nest. This frequently fierce defense was usually in striking contrast to the reaction which the same rat had shown while in the non-maternal state. *c*. Many tests made on maternal rats showed that they would not retrieve small, smooth (clean) corks or a wood-shaving dropped through their cage door as were the proffered pups. *d*. The degree of attentiveness to young by subject rats made maternal in these tests was usually less than that shown by the normal parturient rat. Occasional rats, despite having undergone pregnancy and given birth to a litter, are not maternal at all; in comparison with such mothers our positively responding rats show a wholly different category of behavior. Nest-building also was notably less adequate in our responding and in our "normal reactor" rats than is usual in parturient rats; special tests indicated that this was not wholly due to the short time the pup was left with the subject, since three or more pups left overnight were usually not provided with really good nests. Possibly, however, these latter tests were not suitably timed with the onset of the drive. Nevertheless the hormones which gave positive responses in the present series of virgin or other rats effected a radical change in the unlearned behavior of rats of various sex types. Our all-important criteria of response therefore, though by no means ideal for quantitative study, represent something definable and real.

MATERIALS. The rats used were of Wistar strain. Only virgin females, and males not used as parents, were included in these tests. The rats were fed dog chow and a small amount of cracked corn and hemp once daily; lettuce or cabbage was fed once weekly.

A short characterization of the hormones and extracts used, together with the daily dose of each, follows:

1. Whole anterior pituitary of beef extracted at pH 9–10, 10 (or 5) mgm.
2. Growth preparations (Antuitrin G; Collip; Ayerst, McKenna and Harrison; Evans), 0.2, 0.1 or 0.05 cc. (2 to 6 mgm.).
3. Prolactin, 30 Riddle-Bates units (40 international units: 3–5 mgm.); also 2 smaller dosages (6 R-B u.; 12–24 R-B u.) to control "prolactin content" of some preparations of types 1 and 2 above, and to show effect of dose level.
4. Intermedin (from I. G. Farbenindustrie Gesellsch.), 100 Phoxinus units.

5. LH (Luteinizing, from Doctors Fevold and Hisaw), 2–4 r.u. (57 tests) or 20 r.u. (9 tests).

6. FSH+ thyrotropin, 4 (or 2) mgm.   To stop maternal response, 10 (or 20) mgm.

7. Thyrotropin (+FSH) (From I. G. Farben.), 9 guinea pig units.

8. Adrenotropin, 5 (4 or 2) mgm. (contaminated; see below).

9. Serum of pregnant mare, 0.2 cc. (1.25 x minimum ovulation dose in rabbit).

10. Prolan (Elberfeld, Germany), 15–30 m.u. (or 100 m.u.).

11. Desoxycorticosterone acetate, 0.2 mgm. in sesame oil; or implant of an 8–11 mgm. pellet (only partly absorbed).

12. Progesterone, 0.2 or 0.1 mgm.   13. Testosterone, 0.5 mgm.

14. Estrone, 5 or 50 r.u.; Progynon B, 5–250 r.u. (in 20-day tests, 50–250 r.u.; or pellets in 5 females).   To stop response, 5, 50 or 100 r.u.

15. Cortin (Kendall; Wilson Laboratories), 0.4 cc. (in two doses).

16. Phenol, 0.3 to 0.6 cc. of 0.5 per cent solution.

17. Parathyroid extract (Lilly),[1] 30 units.   18. Thyroxine, 20–50 gamma.

19. Extract (at pH 8.0–9.0) of 20–30 mgm. of desiccated thyroid.

The injection of these preparations only rarely produced abscesses or notable skin reactions.   The volume of oil used to dissolve most steroids was kept between 0.05 to 0.2 cc. per injection since the oil formed subdermal pockets and was slowly absorbed.   Thyroxine injections resulted in loss of body weight.   Phenol and certain FSH+ solutions caused a slight transient local irritation but no obvious change in body weight.

Preparations of types 1 and 2 above affected parental behavior quite similarly; they were also alike in that our assays of them showed them to contain several pituitary hormones (type 1 preparations also contained some posterior lobe hormone).   The results obtained with the two types are therefore summarized together (fig. 1, table 2).   The tests made with two lower dose levels of prolactin were designed in part to indicate the extent to which the prolactin content of the unfractionated pituitary extract (whole A.P.) and "growth" preparations may be expected to affect parental behavior.   Any clearly demonstrated ability of preparations of types 1 and 2 to make rats "maternal" was limited to the normal females and to the higher dosage; and in this case our analysis of the data shows that an undue proportion of normal females were given the highest dosage.

All of the eight different preparations of prolactin used were practically free from FSH and LH as indicated by the following test: the intravenous injection of 50 mgm. of the preparation would not induce ovulation in a 4-month New Zealand rabbit.   Three of these preparations had, and the others had not, considerable ability to enlarge the adrenals of 21-day rats (2-day chicks also used with later preparations) but all these fractions were very similar in their influence on maternal behavior.   About four-fifths of the tests were made with no preservative in the prolactin solution; in the remainder 1.0 per cent butyl alcohol was used.   No difference in the results was detected.   Solutions (pH 8.0) were made up each 4–5 days and kept in the ice-box.   In most of our tests the (salt-free)

prolactin used was heated to 60° C. for 5 hours (or 99° for 1 hr.) at pH 8.0—a procedure which reduced or excluded the possibility that certain pituitary contaminants, and likewise any included enzymes, were responsible for the effect here ascribed to prolactin. None of the other pituitary derivatives used by us was subjected to heat.

The intermedin used was practically free of actions identifiable with other anterior lobe hormones and with pitocin. The LH used was only partially assayed by us; the preparations were not pure LH, but their prolactin content was low. The three preparations of adrenotropin used contained from 0.1 to 1.0 unit of prolactin per mgm.; their content of FSH and thyrotropin was moderate or low; they all had some ability to enlarge seminal vesicles and uteri of 21-day rats.

Preparations of types 6 and 7 were shown by our assays to be essentially similar in that they were quite potent in both FSH and thyrotropin and usually contained smaller amounts of LH, adrenotropin, and posterior lobe hormone. Our own preparations (type 6) contained no more than 0.01 unit of prolactin per milligram, though the donated preparation (type 7) had 0.1 unit per milligram. The Prolan (I.G., Elberfeld) most used was assayed at the source at 300 r.u. per milligram.

Only 5 hypophysectomized, 3 thyroidectomized and a few castrated rats were tested and found non-maternal previous to operation. Other rats were not tested before operation. The number of hypophysectomized animals available for tests of effects of the various hormones was restricted by the high percentage of such animals which became maternal following the operation.

RESULTS. A summary of 2,883 tests made on various types of rats is given in a form suitable for rapid survey and comparison in figure 1. A few additional tests are described in the text. The columns of the graph show only the percentage of positive responses obtained. Since unequal numbers of rats were injected with the different hormones, and since unequal numbers of the different sexual types were injected with the same hormone, it is useful to present additional data in tabular form.

Tests for normal reactors (control). Some rats of all sex types gave positive maternal responses in the preliminary tests ("normal reactors") without any injection of hormone (table 1). In 1045 of the usual 10-day tests it is rather surprising to find that the percentage of such normal reactors is practically equal in males and females. Castration seems to increase slightly the percentage of normal reactors among both males and females. That is indeed the expected result if decrease or suppression of gonad activity is a part of the mechanism by which the maternal response is established.

The results of 110 tests which were continued for an additional 10–30 days indicate that most but not all of the normal reactors were eliminated in the 10-day preliminary tests. These tests therefore show that some rats injected from days 10 to 20 may be expected to become maternal (reactors) quite apart from an influence of the substance injected. We have calculated this number as approximately 15 per cent, and usually only values above 25 per cent may be con-

sidered of significance in relation to the substance injected. About 4 to 5 per cent of all sex types became maternal so long after (4–10 days) a series of injections that the response could not be attributed to excess of the hormone administered.

Since about four-fifths (see above) of hypophysectomized and thyroidectomized rats were not tested prior to operation, and nearly 25 per cent would thus have proved to be normal reactors, it is clear that the "control" data (fig. 1; table 1) suggest an effect of the operation itself which is about 20 per cent greater than is actually the case.

A further effect of castration is reflected in the fact that almost all substances which clearly evoked maternal behavior did this more frequently in castrate than in normal rats of the corresponding sex (fig. 1; table 2). In this list of substances

TABLE 1

Percentage of maternal (parental) responses in control normal rats, and in rats castrated, hypophysectomized or thyroidectomized (uninjected)

DURATION OF TEST	NUMBER OF TESTS	PERCENTAGE RESPONDING (NORMAL REACTORS)			
		Females	Spayed females	Males	Castrate males
Usual 10 days......................	1045	22.6	25.7	21.5	24.6
Hypct. 10 days....................	20	68.8*		75.0*	
Thydct. 10 days...................	25	0.0†		45.4*	
Extended tests—20–40 days**.......	119	26.5	10.0	15.1	23.0
Non-specific responses‡............	859	5.5	4.0	5.1	3.8

* Approximately 20 per cent too high because all "normal reactors" were not eliminated before operation.

† Only 3 tests.

** Non-reactors during 10 days but further tested during 10–30 days.

‡ Positive responses at 4 to 10 days following a last injection but apparently not induced by the treatment.

are: prolactin (30 units), progesterone (in males only), testosterone, desoxycorticosterone, intermedin, LH, phenol, thyroxine and adrenotropin. The only noteworthy exception is whole A.P. extract (for female only) where a disproportionately large number of normal females received the highest dosage. The females of the "20-day estrone" group would appear also to be an exception, but in these rats the maternal response began only 3 to 11 days after the last injection of estrone; these responses are therefore better credited to *withdrawal*, or decrease, of estrone than to its administration.

In general, the positive effect observed in some females (see above) given unfractionated and "growth" preparations could be expected to result from the amount of prolactin which we found to be present in these preparations; but, the absence of effect in normal males, and perhaps in castrates, is of interest in indicating that so-called "growth" preparations (2–6 mgm. daily) do not
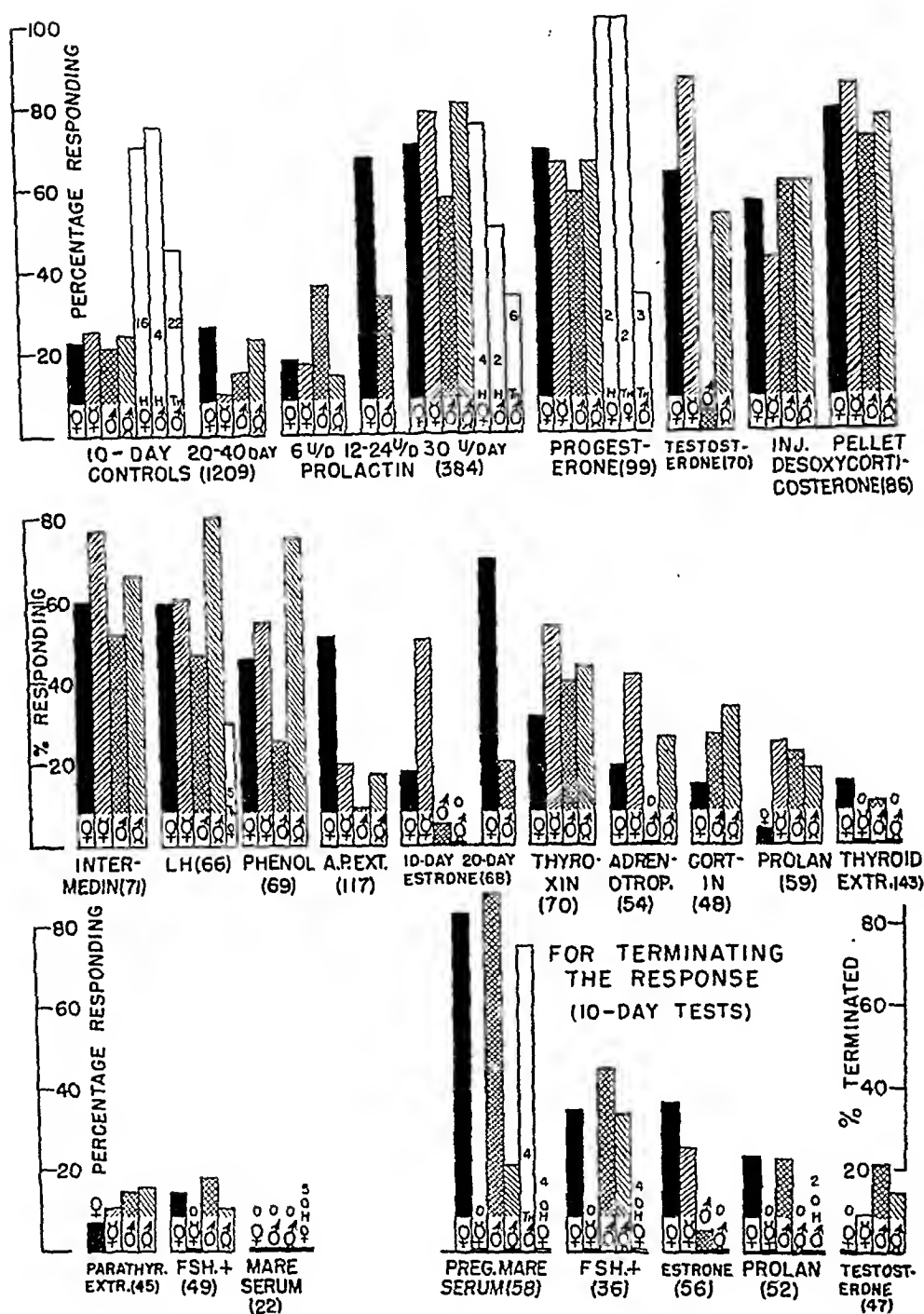
Fig. 1. Graphic illustration of the action of various hormones in exciting or terminating unlearned maternal behavior in rats of various sex types. Conventional signs used for female and male castrates; H = hypophysectomized; Th = thyroidectomized; O = zero effect. Numbers in parentheses or in vertical columns indicate the number of rats tested.

activate maternal behavior. These data (table 2; fig. 1), however, possibly merit further examination. Such unfractionated extracts would seem least likely—as a direct result of the hormones introduced by injection—to stimulate an increased production of any particular hormone by the rat's own pituitary.

It is evident (fig. 1) that increasing the dosage of prolactin from 6 to 30 units daily increased the maternal response in all of the four sex types. At the higher level of dosage, where most tests were made, the results suggest (fig. 1; table 2) that normal males were more resistant than the other three sex types to this action of prolactin. Other data show that those several hormones and substances which arouse parental behavior in significant degree likewise fail more often with normal males than with the other three sex types. In this list, besides prolactin, we find: progesterone, testosterone, desoxycorticosterone (pellet, not injection), intermedin, LH, phenol and whole A.P. extract. Thyroxine is a partial exception. Again, the two agents having highest ability to terminate existing parental behavior, namely, pregnant mare serum and FSH+ thyrotropin, are perhaps *more* effective for that purpose in normal males than in the other sex types. Incidentally, this lower response of normal males under several wholly dissimilar treatments—and an analogous differential response of castrates of both sexes noted earlier—suggest that our tests have been sufficiently numerous and accurate to indicate actual relationship between the response obtained and the substance administered.

The quantitative response to intermedin was definitely below that to prolactin. The four sex types, however, responded curiously alike relative to each other in the two series of tests (fig. 1). The ability of luteinizing hormone (LH) to evoke the maternal response was approximately equal to that of intermedin. Adrenotropin apparently had some ability to evoke the parental response in castrates of both sexes. It had only slight if any potency in normal females and failed completely in 11 tests with normal males. Contaminating substances may have influenced these results.

FSH+ thyrotropin showed little if any ability to initiate the maternal response. It was fairly effective in terminating the response in all groups with the probable exception of castrate females (fig. 1; table 3). Prolan is probably without effect in evoking the parental response; only 11.9 per cent of positive responses were obtained in a total of 59 tests; its ineffectiveness in normal females is clear and definite. It terminated the response in some normal rats but not in castrates. Pregnant mare serum was wholly without ability to evoke the parental response in the three sex types tested (table 2). It terminated the response in about 85 per cent of normal animals and in 3 of 4 tests on thyroidectomized males, but showed little activity in castrates and failed in all of 4 tests on hypophysectomized females (table 3).

Tests with steroid hormones. Desoxycorticosterone acetate has marked ability to evoke parental behavior in all sex types. Its potency, when injected, was about equal to that of intermedin; its potency in the form of implanted pellets, as indicated by a much smaller number of tests, rivalled or exceeded that of prolactin (30 units). In this connection one may recall facts which indicate sex

TABLE 2

Percentage of maternal responses induced by various hormones, extracts and substances in several types of rats

HORMONE OR PREPARATION	FEMALES		SPAYED FEMALES		MALES		CASTRATE MALES	
	Number of tests	Per cent	Number of tests	Per cent	Number of tests	Per cent	Number of tests	Per cent
Anterior pituitary hormones (and pituitary-like)								
Whole ant. pituitary*	48	51.0	18	19.8	30	11.6	21	17.0
Prolactin (30 u.)	109	70.7	33	78.6	54	57.3	36	80.0
Prolactin (in hypct.)	4	75.0			2	50.0		
Prolactin (in thydct.)					6	33.0		
Intermedin	25	60.0	13	77.0	21	52.4	12	66.6
LH (luteinizing)	32	59.4	10	60.0	14	46.4	10	80.0
LH (in thydct.)	5	30.0						
Adrenotropin	18	19.4	12	41.6	11	0.0	13	26.9
FSH+ thyrotropin	21	14.3	7	0.0	11	18.1	10	10.0
Prolan (Elberfeld)	31	3.2	8	25.0	9	22.2	11	18.1
Mare serum	8	0.0			4	0.0	5	0.0
Mare scrum (in hypct.)	5	0.0						
Steroid hormones (from gonad and adrenal)								
Desoxycorticosterone ac.	9	55.5	12	41.7	15	60.0	10	60.0
Desoxycorticosterone (Pellet)	13	77.0	9	83.3	10	70.0	8	75.0
Progesterone	30	68.3	19	65.5	22	59.0	21	65.0
Progesterone (in hypct.)	2	100.0						
Progesterone (in thydct.)	2	100.0			3	33.3		
Testosterone	24	62.5	13	84.6	12	4.1	21	52.4
Estrone (10-day tests)	25	18.0	2	50.0	10	5.0	6	0.0
Estrone (20-day tests)	18	77.8	2	50.0	5	20.0		
Cortin	21	14.3			15	26.6	12	33.3
Other hormones and substances								
Phenol	22	45.8	11	54.5	18	25.0	18	75.0
Thyroxine	21	30.9	15	53.3	19	39.5	15	43.3
Extr. desic. thyroid	20	15.0	8	0.0	10	10.0	5	0.0
Parathyroid extract	15	6.6	11	10.0	7	14.3	13	15.4

* Including "growth" preparations.

hormone production by the adrenals and also atrophic changes in adrenals under dosage with various cortical hormones.

Quantitatively the response to progesterone was only a little below that to 30 units of prolactin, and higher dosage might have proved more effective. Castrates are less responsive to progesterone. Notable, but nowhere tabulated, is the result of treatment of three normal females with a combination of progesterone (0.1 rabbit unit) and estrone (4 r.u.); none of the rats responded to this dosage.

Testosterone has marked ability to evoke parental behavior in all sex types except normal males. The daily dose, 0.5 mgm., would probably produce an abrupt and relatively great increase of circulating androgen (and decrease of gonadotropin and estrogen ?) in the three sex types which responded, but not

TABLE 3

Effectiveness of various hormones in terminating an established maternal response in rats of various types

HORMONE USED	FEMALES		SPAYED FEMALES		MALES		CASTRATE MALES	
	Number of tests	Per cent	Number of tests	Per cent	Number of tests	Per cent	Number of tests	Per cent
Mare serum......................	29	82.7	6	0.0	8	87.5	7	21.4
Mare serum (in hypct.).............	4	0.0						
Mare serum (in thydct.)...........					4	75.0		
FSH+ thyrotropin.	13	34.6	7	0.0	9	44.4	3	33.3
FSH+ (in hypct.).................	4	0.0						
Estrone...........................	18	36.1	18	25.0	9	5.5	11	0.0
Prolan............................	26	23.0	7	0.0	9	22.2	8	0.0
Prolan (in hypct.).................					2	0.0		
Testosterone......................	7	0.0	7	7.2	19	21.0	14	14.3

much *increase* in the non-responsive normal males; indeed, if the brusquely added hormone decreased the male's own production of pituitary gonadotropin his circulating androgen may not have increased at all. This dosage, however, had some ability to terminate the maternal response in normal (and castrate) males only.

Estrone has little ability or absolutely no ability to evoke maternal behavior while injections are in progress. Estrone terminated the established response in some of the two types of females but not in males.

A special and untabulated study of effects of estrogens yielded significant results. Eleven virgin female rats were treated for 20 to 26 days; 5 received estrone in the form of implanted pellets for 20 days, 5 were injected daily with 250 r.u. of estrone in oil for 20 days, and 1 with 100 r.u. of estradiol benzoate for 26 days. Under this treatment the mammaries developed to a degree com-

parable with that found in the later stages of pregnancy; traces of colostrum were present. When 3-day old rat pups were presented to these virgins during the last 3 or 4 days of dosage the pups were given no care. One to 7 days after discontinuance of dosage (pellet removed in 5) such young received full maternal care. In 9 cases the female nursed and reared 1–3 young to weaning; in 2 cases the young were temporarily fed or cared for but not reared. The expression of maternal care as it *first* appeared in these estrogen-treated rats was apparently identical with that which has been used as a criterion of maternal behavior throughout these studies. Since the first behavioral activities were quickly followed by suckling and rearing of young these special tests confirm and justify the interpretation of retrieving and cuddling as expressions of "maternal" behavior.

Cortin showed little or no significant ability to induce the maternal response. This may or may not be associated with low dosage or with the fact that of the steroids injected cortin only was not dissolved in (sesame) oil.

Tests with other hormones and substances. Phenol induced parental behavior in all four sex types; perhaps it was significantly more effective in castrates. Thyroxine exhibited moderate but unquestionable ability to induce the parental response in all sex types. Extract of desiccated thyroid tissue did not clearly prove effective. Parathyroid extract proved ineffective in these tests.

Tests made on hypophysectomized and thyroidectomized rats. Our tests adequately establish the fact that hypophysectomy, or events incident to it, induces or permits a significant percentage of male and female rats to exhibit the maternal response (table 1; fig. 1). Also, a high percentage (4 of 5 tests of rats failing to show this behavior following pituitary removal, and both of 2 males injected immediately after operation) were found to respond to administration of either prolactin or progesterone. A special test was made on 5 females injected with mare serum immediately after pituitary removal; none of these rats became maternal during this 10-day period, though 3 (of 4 survivors) gave the maternal response at 3 to 11 days *after* the injections were stopped (table 2; fig. 1). Finally, mare serum, FSH+ thyrotropin and Prolan apparently have little or no ability (0 of 10 tests) to terminate the established response in hypophysectomized males and females; yet those three agents were potent when tested on unoperated males and females (table 3). This is clear evidence that the pituitary, or a bodily state maintained by the pituitary, is necessary for the *termination* by hormone administration of an established maternal behavior. Moreover pituitary removal alone, unaccompanied by gonadal regression, does not precipitate maternal behavior in female rats. It now appears highly significant that the high incidence of maternal responses in hypophysectomized rats is primarily associated with gonadal regression resulting from loss of support from the pituitary gland.

Tests made on thyroidectomized males show, confirming McQueen-Williams (13), that the operation alone has some ability to evoke parental behavior (table 1). It is notable, however, that male non-reactors, following the operation, seem to respond less well to prolactin and progesterone than do unoperated non-

reactors of any sex type (table 2). Three tests were made for effects of this operation in females; none of the rats exhibited maternal behavior. However, when two of these females were later given progesterone both became maternal. Another group of five thyroidectomized females were non-reactors in their pre-operative 10-day test. Without a subsequent test (in 2 of 5) for the effect of the operation alone they were injected immediately with LH; the group showed a relatively low (30 per cent) response. Thus thyroidectomy was less effective in inducing parental behavior than was hypophysectomy (table 1), and the non-reacting thyroidless male (and female?) rats were also less readily transformed to reactors by prolactin, progesterone and LH (table 2). The response was readily terminated (3 of 4 tests) in thyroidless rats by pregnant mare serum although the latter and FSH+ were ineffective when thus used in hypophysectomized rats.

SPECIAL DATA AND CONSIDERATIONS. *Relation of hypophysectomy and thyroidectomy to the maternal response.* The two preceding paragraphs make it clear that parental behavior may be precipitated and exhibited following surgical removal of either the hypophysis or the thyroid. It should be remembered, however, that these organs had existed and functioned in the responding rats up to the moment of their removal; also that the removal of either of these two organs brusquely sets many or most bodily processes on a new level. Castration also increases both the percentage of reactors (table 1) and the effectiveness of any hormone (fig. 1) which commonly induces parental behavior. It is probable that all of these operations, besides resulting in a loss of sex hormones and a change of hormone balance, either temporarily (anesthesia) or permanently reduce the rate of heat production in these rats. It is conceivable that even a temporary drop in the metabolic rate is favorable for the development of the parental—or perhaps it is more truly a feminine and "maternal"—response. Very important nevertheless is the fact that these several operations leave many or most of the operated rats thoroughgoing non-reactors, and for *these* rats something else is needed—and has been found—to make them "maternal." In this connection it is notable that it has not yet been proved that the anesthesia incident to these operations is wholly without effect on the exhibition of parental behavior. One recalls Ceni's (2) statement that housewives in rural Italy, when in need of a brooder for motherless chicks, simply heavily alcoholize a capon and thereafter successfully turn the job over to him.

We were first to find and report (21) that hypophysectomy may precipitate unlearned maternal behavior (rats). Soon thereafter Leblond and Nelson (11) reported apparently similar observations in mice, though they failed to show that there were any "non-reactor" controls in their mouse colony. The last-named authors concluded that "a nervous mechanism, which may be stimulated without hormonic influences, seems to be the essential factor of maternal instinct in mice and rats. It may be controlled by hormonic factors in normal animals after parturition." The immediately preceding paragraphs of the present paper present much evidence that the pituitary shares importantly in the initiation and in the termination of the maternal response. We regard the precipitation of the parental response by an operation involving pituitary removal as unacceptable

evidence that the pituitary is not causally involved in the development and exhibition of parental behavior. Hypophysectomy (even if done at birth) effects a functional castration of the rat but, as noted above, when the gonads are maintained by injections of mare serum, hypophysectomy fails to result in maternal behavior. In simple hypophysectomy (and anesthesia) gonad depression probably begins at once although prolactin may tend to persist in the blood; prolactin injected subcutaneously in pigeons apparently disappears from the blood only after nearly three days (9).

Special aspects of the maternal response. It is not easy to evaluate the hormonal factors which, apart from any conscious treatment whatsoever, may be involved in the production of about 22 per cent of "normal reactors" in our rat colony. Indeed, under much longer test (and ageing) another 15 to 20 per cent become reactors. This same phenomenon extends to other species, and sometimes to relatively immature individuals of the species. On this point we suggest that the history of effective hormonal participation in the origin and regulation of maternal drive in mammals may extend into the prenatal period. Basophilic cells of the mammalian pituitary produce gonadotropin long before birth, and the automatic (later) dosage of these embryos with the mother's estrogen at or before birth may then cause a temporary repression of fetal gonadotropin, and indirectly of gonadal hormones, along with unequal degrees of "conditioning" for the maternal response in different individuals or litters.

Some difficulty also attends any attempt to find a reason for the failure of response in 20 to 30 per cent of a group of rats which gave 70 to 80 per cent of positive responses under dosage with one or another hormone. Our data show that, in a later test with a different highly effective hormone, the originally refractive 20 to 30 per cent may again yield 70 to 80 per cent of positive responses. Such observations justified the repeated injection, after a 10-day period of rest, of any non-responding rat excepting those that killed the proffered pups. Perhaps an ageing or developmental factor here determined the earlier failure and the later readiness to respond. A few rats that had failed during 10 days to respond to prolactin were injected immediately for another 10 days on a much higher level of dosage (60–100 units) without making them maternal.

Antigonad and related actions of the hormones injected. Much importance attaches to the known or alleged antigonad effects (upon or through either reproductive cycle, gonads, or anterior pituitary) of those hormones which induced maternal behavior in the present tests. Our review of that very extensive literature is too lengthy to publish, and little besides a general statement is possible here. Prolactin causes a temporary suppression (2–4 wks.) of estrous cycles of mice (5), and the same result in the rat was reported by Lahr and Riddle (8) who made the further significant observation that prolactin prolongs the life and apparent functioning of corpora lutea. Two recent studies confirm this latter result and provide more critical evidence that prolactin promotes and maintains the function of the corpus luteum (1, 7).

Besides the information in the literature we have studied (smears, sections of ovary, testis, etc.) many of the rats used in these tests. Though facts from both

these sources are rather inadequate they indicate that, in the dosages and animals used by us, most of the substances which evoked maternal behavior plainly showed antigonad action.   However, for LH particularly (thyroxine, possibly) the term "antigonad" seems inapplicable although it too had a tendency to arrest cycles.   There is definite evidence that most of the effective hormones modify pituitary activity, that prolactin promotes the production of progesterone, and a probability that some of the effective hormones other than prolactin cause an increased output of prolactin by the pituitary.   Estrone, with ability to evoke the response only after cessation of dosage, may have a special type of antigonad action, but it probably modified the output of both gonadotropin and prolactin.

Noble, Kumpff and Billings (14) made significant observations on the ability of corpus luteum hormone and prolactin (also phenol) to induce broodiness in normal and castrate Jewel fish.   Both hormones were highly effective in fish that had previously brooded, and slightly effective in those that had only previous spawning experience, but were wholly ineffective in those that had neither spawned nor brooded.

Richter (15) has shown that an increase in the nest-building activities of hypophysectomized or thyroidectomized rats is there connected with the maintenance of body temperature.   In published comment on a statement by Riddle (18) concerning a part of the present data Richter considered it probable that in our tests the proffered pups were treated as nest-building material, and that the retrieving thus observed is unrelated to the true maternal drive.   In general, Richter suggested that our various effective treatments and operations lowered body temperatures in the treated rats and that their subsequent behavior is not maternal behavior but only a part of the heat regulating mechanism.   This criticism requires consideration.   In our first reported tests (20), conducted at lower temperatures than later, we could distinguish two types of "nest-building" one of which seemed unrelated to the maternal state and the care of young.   Later tests avoided low temperatures although some fluctuations were unavoidable. All nest-building was much less frequent in these later tests; and nest-building, as already noted, is wholly disregarded in all decisions concerning the maternal or non-maternal state of our rats.

As earlier noted it is probable that the temperature factor sometimes has a share in precipitating a change from mating drive to maternal drive; and clearly this might involve, and result from, either a direct antigonad action of extremes of body temperature, or from an indirect effect of temperature upon the output of various pituitary hormones (FSH+, LH, prolactin).   Our study nevertheless provides definite evidence that neither an abnormal environmental nor body temperature is an essential in the production of the behavior which is here called "maternal."   All of the substances tested were used during periods of highest summer heat and at seasons of lower external temperatures, and the typical response to the substances was both initiated and maintained irrespective of season and of a warm or cold room.   Again, although hypophysectomy and thyroidectomy must have reduced the heat production and body temperature of all rats thus operated, only some of them became maternal, and many or most of

the resistant ones could be made maternal by prolactin which probably increased their heat production. In unoperated rats prolactin had no significant effect on body temperature in an average of 50 measurements made on 14 rats (99.2° F.) and 24 concurrent measurements made in 8 uninjected controls (98.9°F.). Hypophysectomy, though it doubtless produced lower body temperature whether uninjected or injected with mare serum, does not precipitate maternal behavior when the latter substance is used to prevent an incidental atrophy of the ovary. Again, it can not be assumed that our thyroxine administrations reduced heat production in normal and castrate rats which they made maternal and on whom a concurrent loss of body weight was observed.

It was repeatedly shown that rats made maternal by these tests would re trieve and cuddle not only young rats but other young. The motion picture film made and exhibited by us at various scientific meetings showed a virgin rat re trieve in succession all of the following offerings: 5 young mice, 4 newly hatched pigeon squabs, 5 young rats. In a few special tests the retrieving and care of young following prolactin was observed to continue for more than two months after the last injection.

Discussion. Of the various theories hitherto proposed concerning the mechanism of activation of sexual and maternal instinct, Lashley's (10) recent review rather clearly indicates that, despite absence of direct evidence, the effective hormones act upon the central nervous system to increase the excita bility of the sensorimotor mechanism specifically involved in the instinctive activity. Many parallel instances of a restricted action of drugs upon localized neural structures (strychnine) and upon psychological functions (mescal) are, however, well known. The results of the present study permit the view that in the rat more than one hormone may be capable (though one only may normally function) of stimulating this specific sensorimotor mechanism, and that by chance a drug (phenol) has been observed to have either a similar selective stimulating action or ability to increase the output of an effective hormone.

Details of this subject need not obscure its broader aspects. Behavior is a child of growth, as is bodily form; and in higher animals growth is the offspring of genes and of hormones. The laying down of structure adequate for behavioral (nervous) mechanisms is an organismal process conducted in a field where both hormonal and neural regulation concurrently function. Constitution thus provides not only for simple reflexes but for innate unlearned behavior. The es sential rôle of hormones in recurrent instinctive sexual and maternal behavior is what may be expected from the predominant rôle of hormones in the regulation of irregularly rhythmic processes generally. Again, from the standpoint of basic similarities of constitution in the two sexes it is far from surprising that a neural mechanism for sexual and for maternal behavior exists in both sexes.

It is well recognized that the mating drive is aroused—more directly—by the sex hormones. In the normal animal this means the concurrent production of gonadotropin and sex hormone. The genetic relationships and differences be tween mating drive and maternal drive are brought into clearer relief by the present study (fig. 2); notable, however, is the earlier observation (16) that birds

which probably never developed any trace of a gonad exhibited pronounced mating and broody behavior. Perhaps the adrenals produced significant amounts of sex hormones in these latter cases and in many castrates also. In both of these drives it is of course the last hormone of the series that is of very special significance. It was elsewhere noted (17) that of the organism's total equipment of drives or instincts it is the maternal drive which appears latest in the life history. This drive would seem to have no significance apart from adult life, or indeed until after pregnancy (mammals) or egg production (other vertebrates). With recurring pregnancies it should and normally does recur. Maternal drive normally disappears in the mating intervals; and the data presented here indicate that an increase of the homologous sex hormone (estrone or testosterone)—resulting from an increase of pituitary gonadotropin—beside promoting mating behavior definitely inhibits or terminates the maternal drive.

ORDER OF HORMONE ACTION IN:

(a) Mating Drive

GONADOTROPHIN ⟶ { ESTROGEN OR ANDROGEN

(b) Maternal Drive

GONADOTROPHIN ⟶ { ESTROGEN ⟶ PROGESTERONE ⟶ OR ANDROGEN ⟶ } ⟶ PROLACTIN OR ?
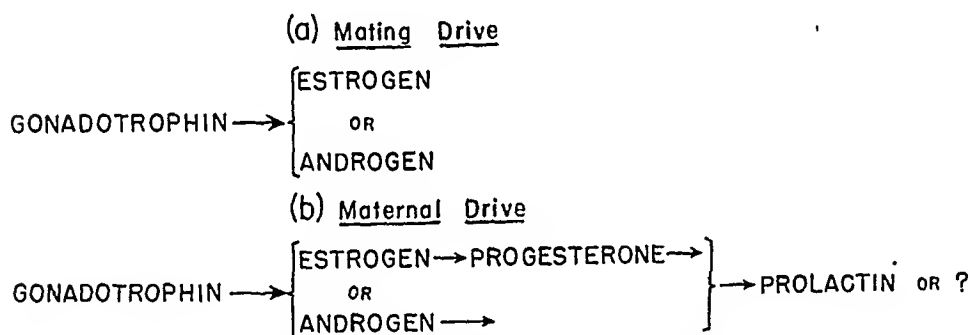
Fig. 2. Relationships and differences between hormonal induction of mating drive and maternal drive.

The normal sequence of nearly contemporary hormonal activity (i.e., apart from early developmental influence) which accompanies either a first or a later exhibition of maternal drive in rats thus both begins and ends with a pituitary hormone. These two hormones—gonadotropin and prolactin—have, however, very unlike properties and functions; broadly, the one is related to building egg and sperm, the other to feeding and care of young.

It is obvious that a study such as is presented in the preceding pages could not have been conducted prior to the time when the several all-essential hormones became available in sufficiently pure form. That time, if it has indeed arrived, was essentially coincident with this study. Because of inter- and contra-actions among the so-called "target" hormones and the pituitary hormones the analysis and interpretation of the results obtained in this prolonged study involves much that is now only partly known. Perhaps several, or even all, of the effective substances have in common the ability to increase the output of one or another "key" hormone—the latter (e.g., prolactin, progesterone, desoxycorticosterone, or a now unknown substance) being more directly concerned in initiating the

maternal drive.   The time for a satisfactory appraisal of the various possibilities spreads into the future.

Though the above statement seems necessary to an interpretation of our experimental results it must not be overlooked that recurrent maternal behavior in the reproducing animal (which the rats in our tests were not) probably depends upon only such hormone or hormones as are in fact released in increased amounts at the right *time* in each reproductive cycle; and, whatever is *thus* released probably must also depress or restrain sex hormone production by the gonad, and then do still *something else* that is auxiliary or necessary.   Extremely few hormones now appear to fulfill these three requirements.   In many mammals testosterone, progesterone and LH activities would seem to be on the wane at the usual onset of the maternal drive, and there is something incongruous in regarding pure LH as "antigonad."   Nothing is known about a cyclic production of desoxycorticosterone and intermedin, and a suitable cycle for either seems improbable.   Prolactin seems in best fit, and it has been proved effective in all species and sex types hitherto tested—fish, doves, fowl and rats.

SUMMARY AND CONCLUSIONS

More than 2900 tests were made with 19 different hormones or hormonal preparations to determine the rôle of hormones in the exhibition of unlearned maternal behavior in rats.

Intact rats aged 60 to 70 days were tested especially for retrieving and cuddling responses daily for 10 days, and about 22 per cent of "normal reactors" were thus separated from the non-reactor rats thereafter subjected to hormonal injection for 10 days with daily records of behavior extending over a further 10-day period. All hormones were tested on males and females and on castrates of both sexes.

In responding rats whose mammaries had been developed with estrone during 20 days the validity of the criteria of response was attested by the ability of these rats actually to feed and rear young.

The maternal drive was initiated in large or in significant numbers of rats of all sex types during injection (or pellet implantation) with various hormones—prolactin, progesterone, desoxycorticosterone, intermedin, LH, phenol and thyroxine; testosterone failed only in normal males.   The drive was not activated by injection with FSH, "growth" hormone, adrenotropin, Prolan, pregnant mare serum, parathyroid extract nor by a special extract of thyroid tissue.

Most but possibly not all of the effective substances rather clearly exerted an antigonad action on the intact rats; the term, antigonad, seems inapplicable to LH although it too had a tendency to arrest estrous cycles.   Castration alone slightly increased the number of "normal reactors," and also increased the effectiveness of all substances capable of exciting the maternal response.

Intact males were as likely as intact females to be "normal reactors," but nonreacting males were more resistant than (the slightly smaller) females to the maternalizing action of all the effective hormones.

Pregnant mare serum, FSH+ and estrone all had much or appreciable ability to terminate well-established maternal behavior in most sex types.   These sub-

stances do not exert this action in rats deprived of their hypophyses, but they are effective in the absence of the thyroid.

Hypophysectomy alone precipitates maternal behavior in approximately 50 per cent of otherwise intact rats, but not if a decrease of the ovarian function is prevented by injections of pregnant mare serum. Non-reactors following hypophysectomy can, in most cases, be made maternal with either prolactin or progesterone.

In male rats thyroidectomy alone excites maternal behavior in about 25 per cent of such tests. Male rats not made maternal by this operation seem thereafter more resistant than normal rats to the positive action of prolactin, progesterone and LH.

The degree of purity or contamination of the various pituitary preparations used was ascertained. Progressively larger doses of prolactin—6, 18 and 30 units—progressively increased the percentage of rats of all sex types made maternal by treatment.

Recurrent maternal behavior in the reproducing animal (which the rats in these tests were not) probably does not depend upon the several hormones here found to be directly or very indirectly active, but upon that one of the group which a, is released in increased amount at the right time; b, exerts an antigonad action, and c, then directly or indirectly increases the excitability of the sensori-motor mechanism specifically involved in this instinctive behavior. Though present information is inadequate the hormone which apparently best fits these requirements is prolactin.

Genetic relationships and differences between mating drive and maternal drive seem clearer in the light of results of this study. The mating drive, or perhaps merely an unexpressed basis for that drive, is apparently an intimate or necessary precursor of the maternal drive. It thus appears that the maternal drive is remotely conditioned by the pituitary hormones (gonadotropins) which govern the building of egg, sperm and sex accessories, and is probably precipitated and regulated by another pituitary hormone (prolactin) whose actions relate broadly to the feeding and care of young.

REFERENCES

(1) Astwood, E. B. Endocrinology 28: 309, 1941.
(2) Ceni, C. Arch. Svizzero di Neur. e Psichiatria 21: 131, 1927.
(3) Ceni, C. Proc. 2nd Internat. Cong. Sex Res., Oliver and Boyd, 286, 1930.
(4) Ceni, C. Arch. Svizzero di Neur. e Psichiatria 42: 209, 1938.
(5) Dresel, I. Science 82: 173, 1935.
(6) Ehrhardt, K. Klin. Wchnschr. 8: 2044, 1929.
(7) Evans, H. M., M. E. Simpson, W. R. Lyons and K. Turpeinen. Endocrinology 28: 933, 1941.
(8) Lahr, E. L. and O. Riddle. Proc. Soc. Exper. Biol. and Med. 34: 880, 1936.
(9) Lahr, E. L., L. H. Elwell and O. Riddle. Arch. Intern. Pharmakol. and Therap. 65: 278, 1941.
(10) Lashley, K. S. Psychol. Rev. 45: 445, 1938.
(11) Leblond, C. P. and W. O. Nelson. This Journal 120: 167, 1937.
(12) Leblond, C. P. J. Genet. Psychol. 57: 327, 1940.
(13) McQueen-Williams, M. Science 82: 67, 1935.

(14) Noble, G. K., K. F. Kumpf and V. N. Billings.   Endocrinology 23: 353, 1938.
(15) Richter, C. P.   Cold Spring Harbor Symp. Quant. Biol. 5: 258, 1937.
(16) Riddle, O.   Brit. J. Exper. Biol. 2: 211, 1925..
(17) Riddle, O.   Proc. Am. Phil. Soc. 75: 521, 1935.
(18) Riddle, O.   Cold Spring Harbor Symp. Quant. Biol. 5: 218, 1937.
(19) Riddle, O., R. W. Bates and E. L. Lahr.   This Journal 111: 353, 1935.
(20) Riddle, O., E. L. Lahr and R. W. Bates.   Proc. Soc. Exper. Biol. and Med. 32: 730, 1935.
(21) Riddle, O., E. L. Lahr and R. W. Bates.   Carnegie Inst. Wash. Year Book 35: 49, 1936.
(22) Riddle, O., E. L. Lahr and R. W. Bates.   Carnegie Inst. Wash. Year Book 39: 221, 1940.
(23) Stone, C. P.   Endocrinology 9: 505, 1925.
(24) Wiesner, B. P. and N. M. Sheard.   Maternal behavior in the rat.   Oliver and Boyd, London, 1933.

SOME PHYSIOLOGIC RESPONSES OF WOMEN AND MEN TO MODERATE AND STRENUOUS EXERCISE: A COMPARATIVE STUDY

ELEANOR METHENY, L. BROUHA, R. E. JOHNSON AND W. H. FORBES

With the technical assistance of F. CONSOLAZIO and W. HOLMES

From the Department of Hygiene and Physical Education, Wellesley College; the Fatigue Laboratory, Harvard University; and the Grant Study, Hygiene Department, Harvard University

Numerous investigators have reported on changes in heart rate, blood pressures, blood sugar, blood lactate, ventilation and oxygen consumption during and following exercise of various kinds, intensity and duration. The typical physiologic responses of normal males to mild, moderate and strenuous exercise have been fairly well established (1–16). Similar observations on young women may be found scattered through the literature (16–27). The physiologic responses of women to exercise are, as might be expected, similar in nature to those reported for young men. At present, however, the literature does not yield sufficient comparable data on the two sexes to permit generalizations as to how such responses differ in degree for work of a given intensity.

It is the purpose of this paper to present and compare data relative to ventilation, oxygen consumption, R.Q., blood lactate, blood sugar, pulse rates and blood pressures for healthy young women and men during and following non-exhausting and exhausting work performed under identical conditions. Since certain of these variables are related to physical fitness for exertion (13, 14) such data should be useful in attempting to evaluate the relative fitness of men and women for this type of work.

Subjects. The seventeen women used in these experiments were graduate students in hygiene and physical education between the ages of twenty and twenty-seven. They do not represent a random sample of young women, since they are selected as to health and probably engage in more physical activity than the average young women in this age range. They should not, however, be considered athletes in a trained state.

The thirty men subjects were between the ages of nineteen and twenty-three. They were drawn from the 250 cases which comprised at the time the Grant Study at Harvard University. None were athletes in training. Ten cases were chosen at random from those designated as "good," ten from "average" and ten from the "poor" classification. The basis of classification has been described and certain data for these same individuals presented elsewhere (13, 14).

PROCEDURE. The moderate exercise, referred to as "the walk," was walking at 3.5 miles per hour on an 8.6 per cent grade for fifteen minutes on a motor driven treadmill. The strenuous exercise, referred to as "the run," was running

at 7 miles per hour on the same grade for five minutes or until unable to continue. Only nine subjects, all men, were able to continue this work for five minutes. Heart rates were measured continuously from the beginning to the end of the experiment with a recording Guillemin cardiotachometer, as described by Cotton and Dill (30). Blood pressures were obtained with sphygmomanometer and stethoscope. Blood sugar was measured on a sample of capillary blood by the method described by Edwards (28). Ventilation and oxygen consumption were determined by the open-circuit gasometer method.

No data on women were collected during the subject's menstrual period, the week preceding or the three days following the period.

The women subjects arrived at the laboratory six hours after breakfast, with no lunch. The typical experiment proceeded as follows:

1:30–2:00 Bed rest
2:00–2:15 Resting ventilation and oxygen consumption measured
2:15–2:25 Sitting and standing heart rate and blood pressures measured
2:25–2:40 Subject walked on treadmill
        2:34–2:39 expired air collected in Tissot spirometer
        2:35   blood samples from finger for sugar and lactate analyses
2:40–2:50 Subject seated beside treadmill
        2:41, 2:43, 2:45, 2:47, 2:49 blood pressure taken
2:50–2:55 Subject ran on treadmill for five minutes or until unable to continue. Expired air collected for each half minute
2:55–3:10 Recovery from run, subject seated beside treadmill
        2:56, 2:58, 3:00, 3:03, 3:05, 3:13, blood pressures taken
        3:00, 3:05, blood sample taken for sugar and lactate analyses

The experiments on men differed only in that they were performed at various times during the day, and the bed rest and measures of ventilation, oxygen consumption, heart rate and blood pressures at rest were omitted.

The "Recovery Index" (13) and the "Work Index" (14), designed to measure physical fitness for exertion, were computed for each subject as follows:

Recovery Index—Walk

$$\frac{300}{\text{recovery pulse rates } (30'' \text{ to } 90'') + (2' \text{ to } 3') + (5' \text{ to } 6')} \times 100$$

Recovery Index—Run

$$\frac{\text{Duration of run in seconds}}{\text{recovery pulse rates } (30'' \text{ to } 90'') + (2' \text{ to } 3') + (5' \text{ to } 6')} \times 100$$

Work Index—Walk

    150 (maximum pulse rate + maximum lactate concentration)

Work Index—Run

    Duration of run in seconds (maximum pulse rate + maximum lactate concentration)

For each index the higher scores represent greater fitness for exertion. The derivation of these indices is described in the references cited.

TABLE 1

Means and extremes of values for selected physiologic variables for 17 women and 30 men performing moderate and strenuous exercise

	MEANS		EXTREMES			
	Women	Men	Women		Men	
Walk (3.5 m.p.h. on 8.6 per cent grade for 15 min.)						
Ventilation, cc./min./kgm..	610	552	467	831	421	748
O₂ consumed, cc./min./kgm. (9–14 min.)...............	27.8	29.6	25.3	30.2	24.5	38.3
R.Q.—maximum...........	0.91	0.89	0.84	0.99	0.56	0.97
Blood lactate, mgm. per cent	40	21	26	58	9	38
Blood sugar, mgm. per cent	115	112	88	141	89	131
Pulse rate—1 min.........	152	140	134	177	112	162
Pulse rate—3 min.........	168	143	150	190	115	168
Pulse rate—maximum......	179	151	156	200	120	172
Recovery pulse—1 min.....	139	116	111	172	85	162
Recovery pulse—2½ min....	123	107	93	141	82	135
Recovery pulse—5 min.....	112	91	82	143	75	124
Systolic pressure—1 min....	148	148	130	160	122	176
Diastolic pressure—1 min...	84	74	76	98	60	90
Recovery Index...........	81	96	67	101	75	120
Work Index...............	−69	−22	−106	−36	−59	14
Run (7 m.p.h. on 8.6 per cent grade until exhausted)						
Duration (sec.)...........	108	216	70	186	105	300
Ventilation, cc./min./kgm. (max.)..................	975	1112	716	1220	690	1400
O₂ consumed, cc./min./kgm. (max.)..................	40.9	51.3	29.6	47.5	30.5	60.5
R.Q.—maximum..........	1.06	1.14	0.72	1.30	0.81	1.45
Blood lactate, mgm. per cent (max.).............	112	119	69	144	68	178
Blood sugar, mgm. per cent (max.).............	156	144	113	235	114	194
Pulse rate—1 min.........	188	177	170	202	157	192
Pulse rate—maximum......	197	194	181	206	178	210
Recovery pulse—1 min.....	163	158	145	180	128	180
Recovery pulse—2½ min....	132	124	111	151	102	148
Recovery pulse—5 min.....	116	114	105	137	95	136
Systolic pressure—1 min....	163	181	140	190	140	212
Diastolic pressure—1 min...	88	77	78	108	48	96
Recovery Index...........	26	55	16	40	28	92
Work Index...............	−201	−95	−266	−132	−208	50

The data. The means and extremes for the data obtained for the seventeen women and thirty men are shown in table 1.

A. *During and following the walk.* Non-exhausting exercise in which all subjects performed the same amount of work per kilogram body weight for

the same length of time, and in which all subjects reached an approximately steady state within the first five or six minutes.

1. There is no marked difference between the men and women in ventilation, oxygen consumption, R.Q. or blood sugar.

2. The women show a more rapid increase in heart rate and reach a higher maximum, although oxygen consumption in cc./min./kgm. is roughly equivalent for the two groups.

3. The rates of recovery for heart rate are approximately the same, although the women must recover from a higher maximum level.

4. The lactate concentration is higher for the women, suggesting a greater degree of fatigue resulting from the same amount of non-exhausting work.

5. Systolic pressures are the same, but diastolic pressure following the walk is greater for the women. (In interpreting the results of these experiments no great significance may be attached to differences in observed blood pressures. It is difficult to obtain accurate readings of blood pressure immediately following exertion; the conditions in the laboratory increased this difficulty; and different observers took the measurements on the two groups. The pressures change very rapidly during the recovery period and a difference of a few seconds in time elapsing before obtaining the measurement may have a marked effect on the reading.)

6. Both indices of fitness are lower for the women.

B. *During and following the run.* Exhausting exercise in which the amount of work per kilogram body weight per second was the same for all subjects, but the duration of the work was determined by the ability of the subject to continue. No subject achieved a steady state at this level of activity. .

1. The average duration of the run for the women is only half that for the men, so that the women performed only half as much work before becoming exhausted.

2. The maximum pulse rate is approximately equal for the two groups, but the women reach this maximum more quickly. The rates of recovery are about the same.

3. The maximum lactate concentration is approximately the same for both groups, indicating that both were equally fatigued, although the women ran only half as long.

4. The men have a higher R.Q., and greater maximum ventilation and oxygen consumption in cc./kgm./min., although maximum heart rates are about the same.

5. The women show a higher blood sugar level after the run.

6. Systolic pressure is lower and diastolic pressure higher for the women immediately after the run. (See note under A–5 above.)

7. Both indices of physical condition are lower for the women than for the men. This difference is greater for the run than for the walk.

8. In all variables there is considerable overlap in the ranges for the two groups.

The extensive overlap between the men's and women's data and the diffi-

culty of comparing data from the run, in which the women performed only half as much work as the men, suggested a second type of comparison. Using a composite of the four indices of fitness for exertion, the eight "best" women

TABLE 2

Means and extremes of values for selected physiologic variables for 8 "best" women and 10 "poor" men performing moderate and strenuous exercise

	MEANS		EXTREMES			
	Women	Men	Women		Men	
Walk						
Ventilation, cc./kgm./min.	544	574	467	623	462	748
O₂ consumed, cc./kgm./min.	27.3	29.9	25.3	30.0	24.6	38.3
R.Q. (max.)................	0.90	0.85	0.87	0.91	0.56	0.97
Blood lactate, mgm. per cent	34	25	26	53	19	37
Blood sugar, mgm. per cent	114	110	102	135	89	126
Pulse rate—1 min.........	148	149	136	164	116	162
Pulse rate—3 min.........	165	151	150	183	122	168
Pulse rate—maximum......	172	159	161	183	130	172
Recovery pulse—1 min.....	130	128	111	154	105	162
Recovery pulse—2½ min....	116	117	93	141	96	135
Recovery pulse—5 min.....	104	107	82	132	95	124
Systolic pressure—1 min....	149	149	130	160	138	168
Diastolic pressure—1 min...	83	72	76	99	60	88
Recovery Index...........	86	87	71	101	75	102
Work Index..............	−56	−34	−83	−45	−59	6
Run						
Duration (sec.)...........	133	138	87	186	105	175
Ventilation, cc./kgm./min. (max.)..................	1000	1055	716	1220	756	1400
Max. O₂ consumed, cc./ kgm./min. (max.)........	41.6	48.7	36.5	47.5	36.2	57.9
R.Q.—(max.).............	1.13	1.10	0.91	1.30	0.81	1.45
Blood lactate, mgm. per cent	112	113	69	144	83	153
Blood sugar, mgm. per cent	155	138	131	200	114	194
Pulse rate—1 min.........	184	180	170	194	165	192
Maximum pulse rate.......	194	194	181	202	187	205
Recovery pulse—1 min.....	164	159	150	180	140	170
Recovery pulse—2½ min....	131	123	117	151	102	136
Recovery pulse—5 min.....	115	113	105	137	96	130
Systolic pressure—1 min....	166	183	140	190	168	210
Diastolic pressure—1 min...	88	83	78	98	66	96
Recovery Index...........	32	35	22	40	28	42
Work Index..............	−174	−169	−132	−197	−143	−208

were selected, for the purpose of comparing them with the ten men classified as "poor." The means and extremes for these two groups are shown in table 2.

It may be observed that for these two groups doing the same amount of work per kilogram of body weight the physiologic responses are very similar. During

and following the walk, in which neither group is pushed to maximal exertion and both attain a steady state, it is noteworthy that:

1. The blood lactate is slightly higher for the women.

2. The maximum pulse rate is higher for the women, but their recovery is prompt, so that recovery pulse rates at one minute, $2\frac{1}{2}$ minutes, and 5 minutes are equivalent for the two groups.

3. The "Work Index" is slightly lower for the women because of the higher lactate concentration and pulse rate. This suggests that these women are less fit than the men for long-continued sub-maximal work. In other words, this work is more strenuous for the women than it is for the men.

In the run, which is sufficiently strenuous to exhaust both groups in about two minutes, and in which both are pushed to their maximum level, these differences disappear. The only differences are:

1. Oxygen consumption in cc./min./kgm. is lower for the women, indicating a slightly higher mechanical efficiency in the performance of the work.

2. Blood sugar is slightly higher for the women.

DISCUSSION. Among the physiologic responses to exercise which differentiate the trained from the untrained (1–10) may be listed:

1. More economical ventilation during exertion

2. Ability to attain a greater maximum ventilation

3. Greater mechanical efficiency as measured in terms of lower oxygen consumption for a given amount of external work

4. Ability to attain a greater maximum oxygen consumption

5. Lower gross R.Q. during exercise

6. Lower blood lactate for a given amount of exercise

7. Ability to push self to a higher lactate before exhaustion

8. Less increase in pulse rate for sub-maximal exertion

9. Quicker recovery in pulse rate following activity

An examination of the data for the thirty men and seventeen women presented in table 1 shows these same differences existing between the men and the women. For the walk, in which all subjects did the same amount of external work per kgm. body weight, the men exhibit lower ventilation in cc./min./kgm.; their blood lactate is lower; and their pulse rate is lower, the maximum rate reached being equal to that attained by the women after only 1 minute of walking. The data indicate throughout that this exertion is more strenuous for the women than it is for the men, even though both are able to achieve a steady state. Stated differently, the men are in better physical condition for this sub-maximal type of exertion and the level at which they reach a steady state is somewhat lower. This is shown also by the differences in the Recovery Index and the Work Index.

Comparisons of data for the run are made difficult because the men performed on the average twice as much work as the women. The data should yield some explanation of why the men were able to continue twice as long before exhaustion. It may be seen that they were able to reach a greater ventilation, higher R.Q., and markedly greater maximum oxygen consumption and

were therefore able to carry on for a longer time. In so far as blood lactate serves as a measure of fatigue, there is little evidence that the men pushed themselves nearer to exhaustion, since the blood lactates are roughly equivalent, but the men ran twice as long before reaching their maximal lactate. The means and extremes for maximum heart rate are almost identical, but the women reached this maximum in half as much time. This more rapid increase is shown in the difference in rate at 1 minute. Again it is evident that this exertion is more strenuous for the women than for the men, taxing all the body systems to their limits in a much shorter time. That the women are less fit for this exhausting exercise is also shown by the great differences in the Recovery Index and the Work Index.

The analysis of the means of the data for the seventeen women and thirty men prompts the generalization that women, as a group, are the "weaker sex," being less fit than men for both moderate and strenuous exertion and exhibiting less endurance for this type of activity.

From the comparisons in table 2 which shows data for the eight "best" women and the ten poorest men, it may be seen, however, that such a generalization may be made only about the *average* performances of the two groups, for the responses of the eight "best" women closely approximate those of the ten "poor" men.

In running uphill at a rate which does not permit the attainment of a steady state by any of these subjects, the eight "best" women and the ten "poorest" men are exhausted in the same amount of time. The women equal the men in maximum ventilation, oxygen consumption, R.Q., blood lactate, increase in pulse rate, maximum pulse rate, rate of recovery and systolic and diastolic blood pressures. The women have a higher blood sugar after the run. This may be due to excitement, or it may be a chance difference. In short, there is nothing to indicate that these eight women are in any way less fit than these ten men for short bouts of exhausting exertion. This is shown also by the similarity of the Recovery Index and the Work Index for the two groups.

In the longer continued but less exhausting activity of walking uphill, the differences are somewhat greater for the eight "best" women and the ten "poorest" men. For the women, pulse rate accelerates more rapidly, and reaches a higher maximum, but recovery during the first minute is also more rapid. This suggests that even these "best" women found this long-continued moderate exertion more strenuous than did the ten "poor" men. This is further shown by the higher blood lactate, which indicates that they were more fatigued. There is no difference in the Recovery Index for the two groups, but the Work Index shows that even the best women are slightly less fit for long-continued submaximal exertion than are the poorest group of men.

Since the walk and the run represent two quite different levels of activity, the statements: a, the groups of eight women and ten men are equally fit for short bouts of exhausting activity; and b, the women as a group are slightly less fit for long-continued activity, are not necessarily contradictory. They do, however, raise the question as to why this difference exists. In the run the

limiting factors are primarily circulatory and respiratory. In the walk, the subjects reached a steady state, in which the circulatory and respiratory systems adjusted to the demands made upon them, but the duration of the activity is such that muscular fatigue becomes a factor. It is possible that differences in muscle strength relative to body weight may provide a partial explanation of why the women were more fatigued than the men by this continued exertion. Data on this point are not available for the present group of subjects.

On the basis of the data presented, it is interesting to speculate on the distribution of physical fitness for exertion in the total population. It is obvious from the data on the Recovery Index and Work Index presented in tables 1 and 2 that there is no clear-cut division between men and women in physical fitness for these two types of exertion. Some of the best women exceed in fitness the poorest men, and for the best half of the women studied and the poorest third of the men the overlap is complete. Thus, it is obvious that in the total population, including both sexes, physical fitness for exertion must be considered a continuous variable, although the distribution is not necessarily normal in form. In this distribution it is evident that the women are more numerous in the "poor" end of the distribution, while the men preponderate at the "good" end, but in the middle of the range the sexes overlap.

It may be pointed out that the subjects of the present experiment do not represent extremes of fitness. The classifications "good," "average," and "poor" are only relative. As has been shown by Johnson and Brouha (13, 14), athletes in training exceed in fitness the "good" men in the present group. The same is probably true for women. As has been stated, the women subjects were selected as to health and amount of daily activity. There are, no doubt, many healthy young women of this age who would make poorer records than the poorest reported here. Similarly, healthy young men have been found who are less fit than the "poor" members of this selected college group. Lacking data on these extremes of performance it is not possible to set the limits of overlap in performance for the two sexes, but it seems probable that it is very great.

SUMMARY

1. Seventeen women and thirty men walked fifteen minutes at 3.5 miles per hour and ran for five minutes or until exhausted at 7 miles per hour on a motor driven treadmill with a grade of 8.6 per cent.

2. Records of heart rate, blood pressure, ventilation, oxygen consumption, R.Q., blood sugar, and blood lactate were obtained.

3. Means and extremes for the data are compared for the two groups.

4. The differences between the averages for the men and women are similar in nature to those between the trained and the untrained.

5. As a group, the women were less fit than the men for both moderate and strenuous exertion.

6. Means and extremes for the data on the eight "best" women and the ten "poorest" men are also compared.

7. The eight "best" women equalled in every respect the performance of the ten "poor" men in the strenuous exhausting exercise. They showed slightly greater fatigue as a result of fifteen minutes of non-exhausting exercise in which a steady state was reached and maintained.

REFERENCES

(1) GEMMILL, C. ET AL. This Journal 92: 253, 1930.
(2) GEMMILL, C. ET. AL. This Journal 96: 265, 1931.
(3) OWLES, W. H. J. Physiol. 69: 214, 1930.
(4) SCHNEIDER, E. C. AND C. B. CRAMPTON. This Journal 129: 165, 1940.
(5) KNEHR, C. A. ET AL. This Journal 136: 148, 1942.
(6) EDWARDS, H. T. ET AL. Travail Humain 8: 1, 1940.
(7) ROBINSON, S. Science 85: 401, 1937.
(8) STEINHAUS, A. H. Physiol. Rev. 13: 103, 1933.
(9) BAINBRIDGE, F. A. The physiology of muscular exercise. Longmans, Green and Co., New York, 1931. (3rd ed. rewritten by A. V. Bock and D. B. Dill.)
(10) DILL, D. B. ET AL. Travail Humain 5: 1, 1937.
(11) ROBINSON, S. Arbeitsphysiol. 10: 251, 1938.
(12) ROBINSON, S. AND P. M. HARMON. This Journal 132: 757, 1941.
(13) JOHNSON, R. E. AND L. BROUHA. Rev. Canad. Biol. 1: 171, 1942.
(14) JOHNSON, R. E. ET AL. Rev. Canad. Biol. (in press.)
(15) ROBINSON, S. ET AL. Human Biol. 13: 139, 1941.
(16) TAYLOR, C. This Journal 135: 27, 1941.
(17) HODGSON, P. Res. Quart. Am. Assoc. Health, Phys. Educ. 7: 3, 1936.
(18) HELLEBRANDT, F. This Journal 101: 357, 1932.
(19) HELLEBRANDT, F. ET AL. Proc. Soc. Exper. Biol. Med. 43: 629, 1940.
(20) Wellesley College Studies in Hygiene and Physical Education. Supp. Res. Quart. Am. Assoc. Health, Phys. Educ. 9: 10, 1938.
(21) BENEDICT, F. G. AND H. S. PARMENTER. This Journal 84: 675, 1928.
(22) HARTWELL, G. AND N. TWEEDY. J. Physiol. 46: ix, 1913.
(23) PROSCH, F. Res. Quart. Am. Phys. Educ. Assoc. 4: 75, 1932.
(24) THOMAS, J. AND A. KEYES. This Journal 129: P 480, 1940.
(25) THOMAS, J. AND G. B. NICHELSEN. (Unpublished data.)
(26) DAMEZ, M. K. ET AL. J. A. M. A. 86: 1420, 1926.
(27) MURPHY, M. A. Res. Quart. Am. Assoc. Health, Phys. Educ. 9: 57, 1940.
(28) EDWARDS, H. T. J. Biol. Chem. 125: 571, 1938.
(29) FOLIN, O. AND H. MALMROS. J. Biol. Chem. 83: 115, 1929.
(30) COTTON, F. S. AND D. B. DILL. This Journal 111: 554, 1935.

THE METABOLIC EFFECTS OF POTASSIUM, TEMPERATURE, METHYLENE BLUE AND PARAPHENYLENEDIAMINE ON INFANT AND ADULT BRAIN

HAROLD E. HIMWICH, ALBION O. BERNSTEIN[1], JOSEPH F. FAZEKAS, HERMAN C. HERRLICH AND EDITH RICH

From the Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, New York

We have previously reported that the rate of utilization of oxygen by the brain of the newborn of various species is less than that of the adult (1, 2, 3). The production of energy by anaerobic processes is also slower in the infant (4). In the present experiments a study was made of the activities of some enzyme systems of adult and infant rat brain. The cytochrome oxidase system was examined with the use of phenylenediamine and glucose dehydrogenase with methylene blue. In addition the cerebral metabolic rates of young and mature rats were compared when the temperature of the environment was altered and the potassium concentration of the suspending medium increased. Potassium was employed because, though it exerts little effect on most tissues, its influence on adult brain is profound; oxidations and aerobic glycolysis are increased while anaerobic glycolysis is diminished (5).

METHOD. Cortical tissues of rats of various ages were minced and suspended in a Ringer's solution buffered with phosphate at pH 7.4 and glucose as substrate. The effect of 0.1 M potassium chloride on the oxygen uptake of these tissues was measured in the Warburg respirometer. These observations included the respiratory quotient of the cortex and the oxygen utilization of the upper portion of the brain stem and medulla. The influence of potassium on the respiratory metabolism of excised cerebral cortex of depancreatized cats was also examined. The respiration of cerebral tissues of rats of various ages was estimated when these tissues were exposed to temperatures of 38°, 40°, and 45° both with and without the presence of 0.1 M potassium chloride. The metabolic rates of the adult and infant brain were compared in the presence of 0.005 N paraphenylenediamine. The anaerobic dehydrogenation time of glucose was studied in Thunberg tubes evacuated of air with 1:5,000 methylene blue as an indicator.

RESULTS. Figure 1 reveals the effect of potassium on the metabolic rate of both infant and adult brain. It may be seen that potassium stimulates markedly the aerobic metabolism of the adult rat cerebral cortex, but has only a small effect both absolutely and on a percentage basis on the infant brain. It should be observed that the response to potassium increases from the time of birth and is similar to that of the adult after 40 days of age. Upper brain stem and medulla are also susceptible to respiratory stimulation by potassium. The

[1] Deceased November 23, 1940.

respiratory quotient of the cerebral cortex is 0.89 without and 0.91 with 0.1 M potassium chloride.   In the presence of potassium the metabolism of the cerebral cortex of a depancreatized cat is also accelerated, the average oxygen utilization rising 53 per cent over a period of one hour.

Increase of temperature is less effective in augmenting the metabolism of infant than of adult brain as seen in figure 2.   The additive response to the combined actions of both potassium and temperature is observed only in the adult, perhaps because the changes in the infant are of such a small magnitude as to be within the experimental error.   The average rate of acceleration of oxygen utilization in the presence of phenylenediamine by the infant brain (average of 12 observations) is 18 per cent during the first 10 minutes and rises gradually to 73 per cent from the 50th to the 60th minute.   This is in contrast to the effects on the respiratory metabolism of the adult brain (average of 12 observa
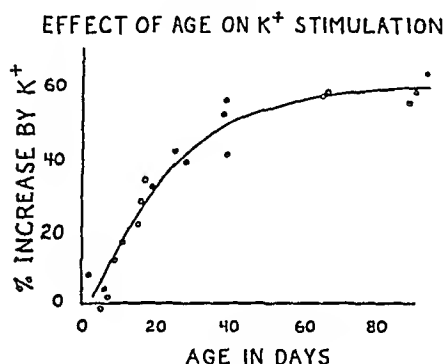
Fig. 1
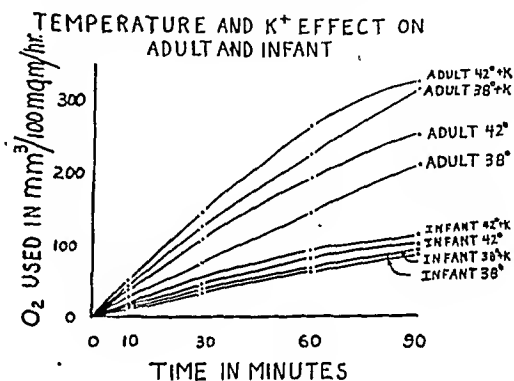
Fig. 2

Fig. 1. Represents the response of cerebral cortex of rats of various ages to potassium. Each point on the curve is the average of at least 10 observations.

Fig. 2. Illustrates oxygen consumption in cubic millimeters per 100 mgm. wet weight of adult and infant brain.   Each point represents the average of 5 observations.

tions) which is increased 124 per cent in the first 10 minutes.   This effect diminishes rapidly so that in the last 10 minutes of the hour the increase over the control is only 38 per cent.   The average decolorization time of methylene blue is 22.3 minutes for 22 adults and 35.4 minutes for 25 infants.

DISCUSSION.   The present investigation explores the relative rates of activity of some enzymatic systems of infant and adult brain in order to determine the underlying mechanisms for their metabolic differences.   In the first place the results present additional evidence that the metabolism of the adult and infant brain are quantitatively different.   This is revealed by the smaller oxygen consumption of the infant cerebral tissues.   The longer sustained rise with phenylenediamine indicates a slower utilization of this substrate by the infant brain.   This result may be ascribed to a lesser activity of the cytochrome oxidase system in the newborn.   The initial stimulation and subsequent depression of adult brain with paraphenylenediamine has been previously noted by Quastel

and Wheatley (6). The observations with methylene blue reveal that the glucose dehydrogenation is slower in the young. The diminished response of these enzyme systems to stimulation may help to explain in part the lower metabolic rate of the infant brain.

An analysis of the differential response of adult and infant cerebral tissue to potassium must take into account the suggestion that growing tissues contain an increased potassium concentration (7). This might tend to diminish the influence of added potassium. If one accepts Dixon's point of view (8), potassium increases the permeability of nervous tissues, then access of substrates to respiratory enzymes is facilitated by potassium. With smaller concentrations of enzymes in the infant brain the effect of potassium would be proportionally reduced. The stimulatory effect of potassium probably increases the carbohydrate oxidation since all parts of the brain oxidize carbohydrate (9, 10). The augmented utilization of oxygen by diabetic cerebral tissue may also be ascribed to carbohydrate oxidation because the brain does not require insulin for this process (11, 12). Finally, the study of the respiratory quotients which remain unchanged by potassium yield additional evidence that the increased respiration is at the expense of the usual cerebral substrates.

The effect of rise of temperature is to increase metabolic rate. The diminished response of the infant brain to elevation of the environmental temperature therefore is similar to the results with potassium. When a greater concentration of potassium is used at increased temperatures, the results are additive. An examination of figure 2 reveals that the combined effects of 0.1 M potassium chloride and a rise in temperature from 38°C. to 42°C. produce an increase of oxygen utilization of 120 mm.^{3} per 100 mgm. of adult cerebral tissue per hour. The sum of the two independent effects (0.1 M potassium chloride = 82 mm.^{3} and increase of temperature 38°C. to 42°C. = 49 mm.^{3}) is 131 mm.^{3} The difference between the two values, 11 mm.^{3}, is within the experimental error.

The observations made in the present investigation may be explained if the concentrations of enzymes are less in the newborn than in the adult. Confirmatory evidence of such a conception is found in the rapidly increasing protein concentration in the brain of the rat during the early neonatal life. It is known that metabolism varies with protein concentration.

SUMMARY

The cerebral tissues of rats of various ages exhibit differences in the response to metabolic stimulants. The oxygen uptake of the infant brain is accelerated to a lesser degree by potassium, increases of temperature, paraphenylenediamine and the decolorization time of methylene blue is delayed. The smaller response to potassium and increase of temperature in the young may be ascribed to a general effect on all enzymes which are of lesser concentration in the newborn, while the slower utilization of paraphenylenediamine and the delayed decolorization time of methylene blue may be accounted for more specifically by smaller concentrations of cytochrome oxidase and glucose dehydrogenase respectively.

REFERENCES

(1) HIMWICH, H. E., Z. BAKER AND J. F. FAZEKAS. This Journal **125**: 601, 1939.

(2) HIMWICH, H. E., P. SYKOWSKI AND J. F. FAZEKAS. This Journal **132**: 293, 1941.

(3) HIMWICH, H. E. AND J. F. FAZEKAS. This Journal **132**: 454, 1941.

(4) FAZEKAS, J. F., F. A. D. ALEXANDER AND H. E. HIMWICH. This Journal **134**: 281, 1941.

(5) DIXON, K. C. Biochem. J. **30**: 1479, 1936.

(6) QUASTEL, J. H. AND A. H. M. WHEATLEY. Biochem. J. **26**: 732, 1932.

(7) FENN, W. O. Physiol. Rev. **20**: 377, 1940.

(8) DIXON, K. C. Brain **63**: 191, 1940.

(9) HIMWICH, H. E. AND J. F. FAZEKAS. Proc. Soc. Exper. Biol. and Med. **30**: 366, 1932.

(10) HIMWICH, H. E., J. P. FROSTIG, J. F. FAZEKAS AND Z. HADIDIAN. Am. J. Psychiat. **96**: 371, 1939.

(11) HIMWICH, H. E. AND L. H. NAHUM. This Journal **101**: 446, 1932.

(12) BAKER, Z., J. F. FAZEKAS AND H. E. HIMWICH. J. Biol. Chem. **125**: 545, 1938.

THE INFLUENCE OF ADRENAL CORTICAL INSUFFICIENCY UPON THE RESPONSE OF MUSCLE TO MOTOR NERVE STIMULATION, WITH PARTICULAR REFERENCE TO THE FIFTH STAGE OF NEUROMUSCULAR TRANSMISSION

HAYDEN C. NICHOLSON, W. Y. TAKAHASHI AND JUAY HONG

From the Departments of Physiology in the Harvard Medical School and the University of Michigan

In a study of the response of a skeletal muscle to continuous tetanization of its motor nerve Luco and Rosenblueth (1939) found that if the stimulation (60 per sec.) was continued sufficiently long, the period of neuromuscular fatigue was followed by a slow but maintained rise of tension which might amount to 60 per cent of the maximal tension developed by the muscle. The initial contraction, the subsequent fatigue and the late rise of tension they referred to respectively as the first, fourth, and fifth stages of neuromuscular transmission. Evidence was subsequently presented (Rosenblueth, Lissák and Lanari, 1939) indicating that the 5th stage was associated with an increased ability of the motor nerve fibers to produce acetylcholine. Since muscular weakness and ease of fatigue are among the most prominent symptoms of adrenal cortical deficiency both in experimental animals and in man, it seemed worthwhile to investigate the possibility of a relationship existing between the activity of the adrenal cortex and the development of the 5th stage. (The fact that the adrenal medulla is not involved was established by the observation of Rosenblueth and Luco (1939) that ligation of the adrenals at the beginning of the experiment does not affect the development of the 5th stage.)

The experiments to be described in this paper were begun during a period which one of us (H. C. N.) spent as a guest in the Department of Physiology in the Harvard Medical School. They were continued after his return to Ann Arbor, in association first with W. Y. T. and later with J. H.

METHOD. All our experiments were performed upon anesthetized cats. The muscles studied were those attaching to the tendon of Achilles (gastrocnemius, plantaris, soleus). The leg was fixed by drills inserted into the tibia. The contractions of the muscles were recorded on a kymograph by attaching the tendon to a myographic lever of the type previously described by Rosenblueth and Luco (1939). The muscles pull against one or more heavy rubber bands, the record indicating mainly changes in tension. Contraction is indicated by an upward excursion of the lever.

Shielded silver electrodes were applied to the popliteal or sciatic nerve after section of the sciatic centrally. The nerve was stimulated at a frequency of 60 per second by thyratron-regulated condenser discharges. Slightly supermaximal shocks were used. The electrode polarity was that which trial showed to be the more effective.

In the original series of experiments performed at Boston dial[1] was used as the anesthetic.   After performing seven control experiments upon normal cats, eight similar experiments were carried out upon cats both of whose adrenal glands had been removed in a single stage operation 24 to 48 hours previously, the animals remaining untreated during this interval.   In many of these latter experiments the difficulty was encountered that even though the animals appeared to be in excellent condition at the beginning of the experiments they failed to survive sufficiently long (3 hrs. or more) to permit the development of the 5th stage.   In an effort to overcome this difficulty a large number of experiments were performed after returning to Ann Arbor in which the use of pentobarbital and urethane as anesthetic agents was compared with dial in both normal and adrenalectomized animals.   These other anesthetics turned out to have no advantage over dial in this respect, though finally a sufficient number of animals survived the requisite time to justify conclusions regarding the influence of the adrenal insufficiency upon the 5th stage.

TABLE 1

TYPE OF EXPERIMENT AND NUMBER OF EXPERIMENTS IN EACH GROUP	AVERAGE HEIGHT OF INITIAL CONTRACTION	AVERAGE TIME REQUIRED FOR TENSION TO FALL TO 25 PER CENT OF THAT DEVELOPED IN INITIAL CONTRACTION	AVERAGE TIME FROM INITIAL CONTRACTION TO BEGINNING OF 5TH STAGE	AVERAGE HEIGHT OF 5TH STAGE IN PER CENT OF INITIAL CONTRACTION
	mm.	*min.*	*min.*	
(7) Control (Boston)................	80.1	5.6	141 (range 90–180)	39.7
(8) Adrenalectomized (Boston)......	97.1	6.0		
(19) Control (Ann Arbor)............	116.5	19.2	153 (range 92–208)	30.1
(8) Adrenalectomized (Ann Arbor)...	118.1	20.9		

RESULTS AND DISCUSSION.   Table 1 summarizes the results of these experiments as regards the first and fourth stages of neuromuscular transmission in control and adrenalectomized animals and as regards the fifth stage in the control animals.   The results of the control experiments were exactly similar to those described by Rosenblueth and Luco (1939).   In three of the adrenalectomized animals the operation was performed 24 hours prior to the experiment. In the remaining thirteen the adrenalectomy was carried out 48 hours before the experiment.   In the Ann Arbor experiments the magnification of the muscular contraction was somewhat greater and the tension against which the muscle contracted was somewhat less than in the Boston experiments, accounting for an apparently higher contraction and slower onset of fatigue in the former series.   The effects of adrenalectomy were the same in the two groups.

It is clear from table 1 that, as indicated either by the height of the initial contraction or the rapidity of onset of fatigue, these animals experimented upon

---

[1] The dial and desoxycorticosterone acetate used in these experiments were kindly furnished us through the courtesy of Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Incorporated.

either one or two days after adrenalectomy were not weak. The actually greater height of the initial contraction in the adrenalectomized animals, slight in the Ann Arbor experiments but more marked in the Boston series, and the slightly slower onset of fatigue in the adrenalectomized than in the normal animals may probably be accounted for by the fact that in choosing animals for adrenalectomy an effort was made to select particularly large, healthy appearing animals.

While adrenalectomy seemed to be without significant effect upon the 1st and 4th stages of neuromuscular transmission it did markedly interfere with the development of the 5th stage. Of the sixteen experiments upon adrenalectomized animals, 5th stages were observed in two. In one, performed 24 hours after adrenalectomy, there developed a 5th stage which was of normal height though less well maintained than normal, its tension declining about 30 per cent in two hours. The greatest fall observed in a similar period in any of the normal ex-
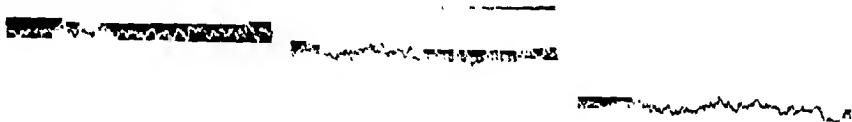
Fig. 1. Illustrating progressive subsidence of 5th stage in cat adrenalectomized 24 hours prior to the beginning of the experiment; 10-minute segments of record separated by 40-minute intervals. The base line indicates the 4th stage (fatigue) level.

periments was about 5 per cent. A portion of the record of this experiment is reproduced in figure 1. In one other adrenalectomized animal a 5th stage appeared. This experiment was performed 2 days after the adrenalectomy. The 5th stage began 87 minutes after the initial contraction. In the next 38 minutes there was an increase in tension amounting only to 3 per cent of that developed in the first stage. The tension then began to fall, the animal dying 18 minutes later.

In the remaining 14 experiments upon adrenalectomized animals no 5th stage was observed. Inasmuch as considerable time is required for the appearance of the 5th stage in normal animals, the duration of these experiments upon adrenalectomized animals is, of course, of significance. The average time of appearance of the 5th stage in 26 control experiments was 147 minutes, with a variation from 90 to 208 minutes. In only 3 of these experiments with the time of appearance of the 5th stage greater than three hours, the time in these experiments being 200, 207 and 208 minutes respectively. The durations of the experiments upon adrenalectomized animals in which no 5th stage appeared

were 80, 85, 100, 102, 159, 160, 160, 200, 210, 270, 280, 300, 330 and 330 minutes respectively. Inasmuch as 6 of these experiments continued beyond the maximal time of appearance of the 5th stage in the control series, and 4 others beyond the average time of such appearance, the conclusion seems inescapable that adrenalectomy may result in failure of the 5th stage of neuromuscular transmission.

Sham operations were performed upon three cats. In these the same anesthetic (ether) was used as in the adrenalectomies, the same incision was made, and the intestines handled somewhat more roughly and for a longer time than was the case during adrenalectomy. The adrenals, however, were not removed. Each of the animals was experimented upon two days after the sham operation. In each case a 5th stage was observed, though the average height of the 5th stage was about 17 per cent of the initial contraction, definitely less than in the control series.

Finally, a number of adrenalectomized cats were given daily intramuscular injections of 0.5 to 2.0 mgm. of desoxycorticosterone acetate (Percorten, Ciba). Seven of these animals were experimented upon 2, 4, 6 and 17 days after adrenalectomy. In one of the seven, 6 days after operation, no 5th stage was observed. In the other 6, the 5th stage appeared at a normal time after the initial contraction, the average height of the 5th stage being 26 per cent of the initial contraction, slightly less than in the control series.

It appears, then, that the failure of the 5th stage to develop following adrenalectomy is the result of the adrenal cortical deficiency itself and not of the trauma incident to the operation, and that the defect can be corrected in large part at least by desoxycorticosterone.

The question now arises as to the site of the failure of the 5th stage. Although the 5th stage has been shown by Rosenblueth and Luco (1939) to be a phenomenon of the neuromuscular junction, its failure to appear might of course result from failure of the muscle, of the junction, or of the nerve fibre. Although the skeletal muscles of adrenalectomized animals may fatigue more readily than normal it seems very unlikely that this could account for the non-appearance of the 5th stage. Muscles stimulated directly are very resistant to fatigue compared to those stimulated through the motor nerve. Furthermore, in experiments such as ours in which the motor nerve is stimulated 60 times a second the tension of the muscle has ordinarily fallen practically to zero within the first half-hour, due to junctional fatigue. Since the muscle is subsequently protected from activity by this junctional fatigue it seems unlikely that the subsequent failure of the tension characteristic of the 5th stage to develop might result from a disturbance in the muscle itself. Nevertheless it was felt that experiments involving lower rates of stimulation might furnish some direct evidence regarding the ability of the muscles of adrenalectomized animals to develop tension. First a series of 5 experiments was performed upon animals adrenalectomized 2 days previously, the stimuli being applied to the sciatic nerve at a frequency of 2 per second. The contractions of the gastrocnemius, plantaris and soleus were recorded as before. A normal muscle stimulated in this way

shows little or no fatigue in the first 3 or 4 hours. One untreated adrenalectomized animal lived 216 minutes after the beginning of stimulation at 2 per second. After 3 hours the height of the recorded contraction still amounted to 54 per cent of the original. Shortly later, however, the contractions began to decrease rather sharply, the animal dying about half an hour later. Of the remaining four experiments, one continued only 70 minutes, the tension after 1 hour having declined to 22 per cent of the original. Another continued 131 minutes, the tension after 2 hours having declined to 14 per cent of the original. In the other two, the animals were kept alive over three hours with the aid of intravenous injection of a glucose-gelatin solution, the tension after 3 hours amounting to 2 per cent and 14 per cent respectively.

Another series of four experiments was performed in which a stimulus frequency of 30 per second was used. At this frequency in a normal animal, the tension after 3 hours is usually at least 40 per cent of the original. One experiment performed 2 days after adrenalectomy lasted 210 minutes, the tension after 3 hours amounting to 12 per cent of the original. Another experiment lasted 155 minutes with a tension of 11 per cent of the original just a few minutes before death. In the other two experiments injections of glucose-gelatin were given in order to maintain an adequate blood pressure and prolong the experiment. One of these lasted 161 minutes with a tension of 41 per cent of the original shortly before death. The other lasted 6 hours. The tension after 3 hours was 60 per cent of the original, after 4 hours 32 per cent, after 5 hours 13 per cent and after 6 hours 4 per cent.

In the experiments at 60 per second, after the 5th stage had failed to appear, turning off the stimulating current never resulted in a relaxation of the muscle amounting to more than 2 per cent of the initial contraction. Since in the experiments at slower rates of stimulation a much higher tension was maintained until shortly before death, it appears that the very low tension observed in the 60-per-second experiments did not represent the maximal tension which the muscle itself was capable of developing, and that failure of the 5th stage cannot be attributed to inability of the muscle to contract.

There remain junctional failure and nerve-fibre failure as possible explanations of the non-appearance of the 5th stage in adrenalectomized animals. Considering the much greater susceptibility to fatigue of the neuromuscular junction the former possibility seems the more likely. However, Maes (1937) has shown that adrenalectomy in frogs results in a marked increase in the fatigability of nerve fibres and the possibility must be recognized that such nerve-fibre fatigue might account for the failure of the 5th stage. The final answer to the question must await nerve-action-potential studies in adrenalectomized cats stimulated at 60 per second.

The question may be raised whether the non-appearance of the 5th stage, whatever its site, represents the direct effect of withdrawal of the adrenal cortical hormone or whether it may be secondary to some other bodily change associated with cortical deficiency, e.g., a circulatory inadequacy. Lanari and Rosenblueth (1939) found that extensive dissection about the superior cervical ganglion

resulted in a failure of the 5th stage of ganglionic transmission, a failure which they attributed to "the unavoidable slight impairment of the circulation in the ganglion," suggesting that the 5th stage of ganglionic transmission was rather susceptible to slight alterations in blood supply. We studied the effect of hemorrhage upon the 5th stage of neuromuscular transmission and found that in general the level of the 5th stage varied in the same direction as the blood pressure, and that it disappeared completely when the pressure fell below 50 mg. Hg. This effect is illustrated in figure 2.

In the experiments upon adrenalectomized animals the blood pressure, while it is ordinarily not far from normal at the beginning of the experiment, usually
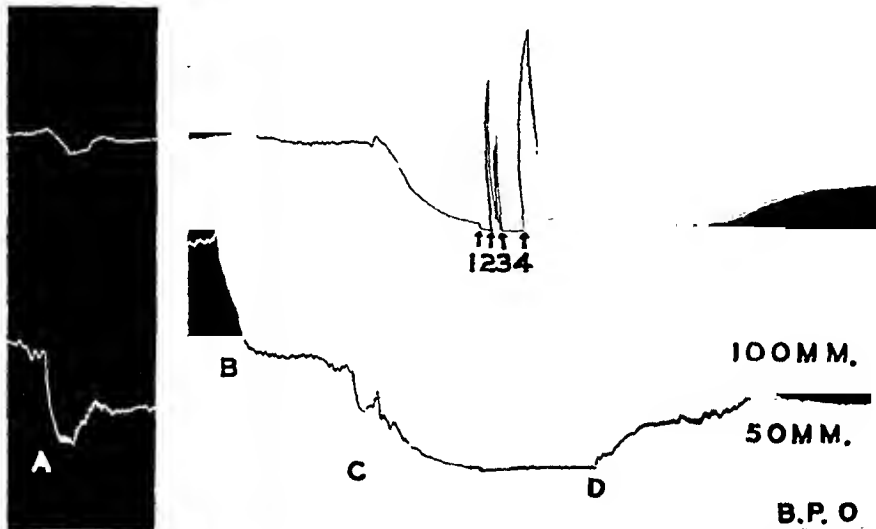
Fig. 2. Illustrating the effects of variations in arterial blood pressure produced by hemorrhage and reinjection upon the level of the 5th stage. Upper record, mechanical response of muscle. Lower record, blood pressure by mercury manometer. At *A* lowering the blood pressure from 106 to 66 mm. Hg lowered the 5th stage level 2 mm. At *B* lowering the blood pressure from 168 to 98 mm. lowered the 5th stage level 1 mm. The 5th stage was abolished by lowering the blood pressure to 30 mm. at *C* and restored by reinjecting defibrinated blood at *D*. At 1 the stimulating current was turned off. At 2 it was turned on again. The muscular contractions at 3 are due to inserting needle electrodes into the muscle. The powerful contraction at 4 is due to direct electrical stimulation of the muscle at a frequency of 60 per sec.

falls to a rather low level within the first 2 or 3 hours. In this connection, it may be significant that in the one experiment performed 2 days after adrenalectomy in which a 5th stage appeared, it began unusually early, only 87 minutes after the initial contraction. Certainly as judged by the blood pressure, the animals would appear to be much better able to support the tension characteristic of the 5th stage one and a half hours after the beginning of the experiment than three hours. Of the 6 experiments involving stimulation at 60 per second which continued 210 minutes or more, in one blood pressure was not recorded. In another, the blood pressure was below 50 mm. the last two and a half hours. In the remaining 4 the lowest blood pressures observed were

52, 56, 83 and 86 mm. Hg. However, in the experiment in which the blood pressure reached 56 mm., the lowest pressure recorded within the first 3 hours was 70 mm. It appears then that in three of these long continued experiments the circulation as judged by the blood pressure level was fairly adequate beyond the maximal time of appearance of the 5th stage in the control experiments. However, an adequate blood pressure does not necessarily mean an entirely adequate circulation, and of course there are other bodily changes resulting from adrenal insufficiency which might conceivably account for the failure of the 5th stage to appear, e.g., alterations in electrolyte concentrations. Further information might be provided by studies involving adrenalectomized animals on a high sodium, low potassium diet.

Finally, it may be asked whether the fact that the 5th stage was obtained in animals treated with desoxycorticosterone indicates that this substance represents a hormone specifically involved in the development of the 5th stage, or whether the 5th stage appeared simply because of the improvement in the general condition of the animals. We are unable to answer these questions.

SUMMARY

The response of muscle to motor nerve stimulation at a frequency of 60 per second was studied in normal anesthetized cats and in anesthetized cats whose adrenals had been removed from 24 to 48 hours previously. At this frequency of stimulation, the height of the initial contraction and the rapidity of onset of fatigue are not significantly different in the normal and the adrenalectomized animals. Such cortical adrenal insufficiency, however, prevents or seriously impairs the development of the 5th stage of neuromuscular transmission. This defect is in large measure corrected by desoxycorticosterone acetate.

Experiments involving stimulation at frequencies of 2 per second and 30 per second show that at these frequencies fatigue occurs more rapidly in the adrenalectomized than in the normal animals, but not rapidly enough to account for the failure of the 5th stage on the basis of contraction fatigue.

The possibility that the failure of the 5th stage to develop may be secondary to other effects of adrenal insufficiency such as a circulatory inadequacy is considered.

REFERENCES

LANARI, A. AND A. ROSENBLUETH. This Journal 127: 347, 1939.
LUCO, J. V. AND A. ROSENBLUETH. This Journal 126: 58, 1939.
MAES, J. Arch. Internat. de Physiol. 45: 135, 1937.
ROSENBLUETH, A., K. LISSÁK AND A. LANARI. This Journal 128: 31, 1939.
ROSENBLUETH, A. AND J. V. LUCO. This Journal 126: 39, 1939.

THE EFFECTS OF TESTOSTERONE PROPIONATE ON RENAL FUNCTION IN THE DOG, AS MEASURED BY THE CREATININE AND DIODRAST CLEARANCE AND DIODRAST TM[1]

C. A. WELSH, A. ROSENTHAL, M. T. DUNCAN AND H. C. TAYLOR, JR.

From the Department of Obstetrics and Gynecology, New York University College of Medicine

Changes in the size of the kidney of laboratory animals after the administration of various steroid hormones have been reported. Korenchevsky and his associates have shown that castration reduces the kidney weight of male rats, that male hormones restore the kidney weight of castrates to normal and that in intact rats, kidneys above the normal weight may be produced by the administration of testosterone (15, 16, 17, 18, 19). The same effects have been demonstrated in mice (23, 24) and the work on rats has been confirmed (20). That the gain is not simply due to water is shown by observations that dry weights are likewise increased (20). It has also been shown that testosterone increases the hypertrophy of the remaining kidney after unilateral nephrectomy (21), diminishes renal atrophy after unilateral ureteral ligation (27), and affords some protection against mercuric chloride poisoning (26).

The renal hypertrophy in response to the androgens appears to be localized chiefly in the tubules, although some glomerular effects have been noted. In rats, both cells and lumina are increased in size (19). In mice, structural changes also occur in the glomerulus, consisting of a replacement of the flat cells of the parietal lamina of Bowman's capsule by cuboidal cells of the type found in the proximal tubules (4, 24). Such cuboidal cell capsules are known to be present in small numbers even in the kidneys of untreated mice, particularly during pregnancy, but their proportion is greatly increased by testosterone injections (5).

Other hormones seem also to have some effect on the kidney, for changes in renal weight have been reported to follow treatment of rats with estrogens (14, 19, 20) and desoxycorticosterone (6, 20). The compensatory hypertrophy of the remaining kidney after unilateral nephrectomy in dogs (37) and in rats (22) is prevented by hypophysectomy.

A decline in sodium excretion and in urine volume occurs in men and women after testosterone administration (10), but this effect is reported to be slight or absent in dogs (34). A diminished excretion of potassium, inorganic phosphorus and nitrogen is, however, a well defined effect of testosterone propionate administration in both man and the dog (10, 11, 12, 13, 34). After discontinuation of androgen administration in man, sodium, chloride and water are rapidly lost; potassium and inorganic phosphorus more slowly, while nitrogen is apparently stored for several weeks (10). The retention of nitrogen and potassium have been interpreted as evidence of the formation of new tissue.

The decreased excretion of sodium and potassium suggest that the androgens may affect also the specific reabsorptive power of the renal tubules. This view seems in accord with the conclusions reached by Durlacher, Darrow and Winternitz (6), who after studying the effects of high and low potassium diets on renal size, suggested that the renal hypertrophy which follows adrenal cortical hormone administration is caused by the increased tubular reabsorption of the potassium ion. The only functional study of tubular activity in relation to endocrine control thus far available is, however, that of White, Heinbecker and Rolf (35, 36) who report that after hypophysectomy in dogs, diodrast Tm is markedly reduced with a concomitant reduction in filtration rate and renal blood flow.

Diodrast Tm (the maximal rate of diodrast excretion by the renal tubules under conditions of saturation) represents one aspect of tubular function which is amenable to precise measurement (30). This paper is a report on the effects of testosterone propionate on this function in the female dog. Observations were also made on the creatinine clearance (filtration rate) and diodrast clearance (effective renal plasma flow) (29). Quantitative methods of evaluating the tubular reabsorption of potassium and sodium are not yet available, and so our observations on these substances have been confined to the total 24-hour urinary excretion rate.

METHODS. Three young female dogs, each of which had had one litter, were used. In each dog a series of control observations was followed by a period during which testosterone propionate was administered daily. Observations were continued during a recovery period after the hormone was discontinued.

Water was given by a stomach tube about one and a half hours before the beginning of the renal clearance periods to insure an adequate urine flow. Creatinine and diodrast were administered intravenously, first in a priming dose then in a constant intravenous infusion with 7.5 per cent mannitol in distilled water. Blood samples were drawn at intervals and plasma levels of creatinine and diodrast for the midpoint of each period were taken from a curve drawn from the observed values. The general level of plasma concentrations in different experiments ranged from 12 to 40 mgm. per cent creatinine, 1.2 to 3 mgm. per cent diodrast during the clearance measurement, and 17 to 57 mgm. per cent during Tm measurement. The blood levels for any one experiment varied little from period to period.

The first urine collection period was begun twenty to thirty minutes after the infusion had been started. Urine was collected by catheter, air being used to empty the bladder at the end of each period. The bladder was washed out with distilled water only in those periods during which the urine flow was low. Three to four urine collection periods were run for creatinine and diodrast -clearances and three to five periods for diodrast Tm.

In one dog the changes in diodrast Tm after testosterone were correlated with alterations in the daily urinary excretion of sodium and potassium. This animal was placed on a standard diet and all urine collected for a seven-week control period and twelve days of hormone administration. The diet consisted of

cracker meal, meat juice powder, yeast, milk powder, vegetable oil, cod liver oil and salt mixture. The food was prepared for one week at a time, carefully mixed, and divided into eight daily portions of equal weight. The eighth portion was analyzed for sodium and potassium each week, the analyses from week to week being found in close agreement. Urine was collected by catheterization twice daily, each 24-hour period ending after the morning catheterization. After the late afternoon catheterization, the pan on the floor of the cage was changed and any urine excreted during the night was collected in a bottle beneath the cage.

Diodrast Tm was calculated as $UV - FWP_DCcr$, where UV is the total rate of diodrast excretion, P_D the plasma level of diodrast, Ccr the creatinine clearance and FW the free, filterable diodrast as read from P_D and plasma albumin in the nomograph of Smith and Smith (31). Plasma creatinine and diodrast iodine were determined on cadmium filtrates (8), creatinine by the Folin and Wu method (7) and diodrast iodine by the Alpert method (1). Plasma albumin was determined by a modification of the Howe (9) method. Sodium was determined in urine and plasma gravimetrically by the uranyl zinc acetate method of Butler and Tuthill (3), the plasma samples being ashed with sulphuric acid in a muffle furnace at 500°C. Potassium was determined by the Brodie and Studenski (2) modification of the Shohl and Bennet (28) chloroplatinate method, and read colorimetrically on an Evelyn photoelectric colorimeter according to Tenery and Anderson (32). Both urine and plasma were prepared for analysis by ashing with sulphuric acid in a muffle furnace at 500 to 550°C.

The dose of testosterone propionate[2] to be used in the dog experiments was obtained by preliminary tests upon mice to find the minimum amount required to produce hypertrophy of the kidneys. It was found that a dose of 0.1 mgm. of testosterone propionate daily for nine days produced the same degree of hypertrophy of the mouse kidney as did a dose of 0.69 mgm. daily for sixteen days. The dry kidney weight in each of these groups was 26 per cent above the kidney weights in the control group. A dose of 0.05 mgm. daily for nine days, however, resulted in an increase of only 18 per cent in dry kidney weight.

To give a dose of testosterone in the dog comparable by weight to that which produced apparently maximal effects in mice would have required about 40 mgm. daily. To allow for possible species differences in susceptibility, 100 mgm. was chosen as a daily dose which might be expected to produce measurable effects. The substance was administered subcutaneously in 4 cc. of sesame oil. The total amounts given in any series of experiments varied from 600 to 2,100 mgm. Renal clearances were determined in the morning before the daily testosterone injection so that twenty to twenty-four hours had always elapsed between the last dose of testosterone and the running of any renal function test

RESULTS. The data on renal clearances, diodrast Tm and clearance ratios. are summarized in table 1 in relation to the duration and amount of testosterone propionate administered up to the time when a particular observation was made.

[2] Crystalline testosterone propionate "Oreton" was supplied through the courtesy of Dr. Max Gilbert of the Sehering Corporation.

TABLE 1

Creatinine clearance, diodrast clearance and diodrast Tm in control periods, during testosterone administration and in recovery periods

DOG	SURFACE AREA	DATE	PERIOD DURING WHICH OBSERVATIONS WERE MADE	TESTOSTERONE PROPIONATE TO DATE	DIODRAST Tm	PLASMA CLEARANCES		EFFECTIVE BLOOD FLOW	FILTRATION FRACTION C_{Cr}/C_D	C_D/T_{mD}	C_{Cr}/T_{mD}	AVERAGE TEMPERATURE DURING T_{mD} DETERMINATIONS
						Creatinine	Diodrast					
	sq.m.			mgm.	mgm. iodine per minute	cc. per minute	cc. per minute	cc. per minute	per cent			°F.
F 1	0.57	June 30, 1941	Control			98.4					3.57	
		July 3, 1941	Control			119.0	685	1026	17.4	24.8	4.31	
		July 7, 1941	Control		27.6	113.0	592	967	19.1	21.5	4.10	
		July 8, 1941	Estrus									
		July 14, 1941	Estrus			97.6	676	1023	14.5	21.1*	3.04*	
		July 17, 1941	Estrus		32.1	108.8†					3.39	
		July 21, 1941	Control		24.1	86.4†					3.59	
		July 23, 1941	Control		23.0	90.0†					3.91	
		July 24, 1941	Testosterone propionate 100 mgm. daily									
		July 28, 1941	Testosterone	400	30.4	88.2	675	1022	13.1	22.2	2.90	
		August 1, 1941	Testosterone	800		93.9	845	1258	11.1	24.9*	2.77*	
		August 2, 1941	Testosterone	900	33.9	79.7†					2.35	100.5
		August 4, 1941	Testosterone	1100		101.4	519	745	19.5	15.2*	2.97*	
		August 5, 1941	Testosterone	1200	34.1	91.0†					2.67	100.5
		October 27, 1941	Recovery		32.3	92.5†					2.87	100.4
		November 5, 1941	Recovery		30.6	92.3	406	717	22.7	13.3	3.02	101.2
		November 7, 1941	Recovery			101.2	416	684	24.4	13.6*	3.31*	
		November 12, 1941	Recovery			93.3	430	676	21.7	14.1*	3.05*	
		November 13, 1941	Testosterone propionate 100 mgm. daily									
		November 19, 1941	Testosterone	600	44.2	98.4	476	771	20.7	10.8	2.23	100.4
		November 24, 1941	Testosterone	1100		109.6	479	768	22.9	11.1*	2.53*	
		November 28, 1941	Testosterone	1500	43.3	92.5	412	636	22.4	9.5	2.14	
		December 4, 1941	Testosterone	2100								
		December 14, 1941	Recovery		27.4	92.6†					3.42	102.2
		December 29, 1941	Recovery		22.9	93.8†					4.10	101.3
		January 9, 1942	Recovery			86.6	375	722	23.1	16.4*	3.78*	
		February 12, 1942	Recovery		28.3	89.3†					3.15	100.8
T 2	0.58	November 14, 1941	Control		17.6	87.7	330	588	26.6	18.7	4.98	102.2
		December 3, 1941	Control		15.8	88.0	349	627	25.2	22.1	5.57	100.4
		December 5, 1941	Control			82.2					5.20*	
		December 8, 1941	Control			76.2					4.82*	
		December 8, 1941	Testosterone propionate 100 mgm. daily									
		December 12, 1941	Testosterone	400	36.9	72.6	360	514	20.2	10.3	2.07	102.2
		December 18, 1941	Testosterone	1000	26.9	67.8†					2.52	101.0
		December 19, 1941	Testosterone	1100								
		December 22, 1941	Recovery		30.3	72.6	310	488	23.4	10.2	2.40	101.2
		January 5, 1942	Recovery		12.5	84.3†					6.76	101.2
		January 22, 1942	Recovery		12.5	89.1†					7.14	101.2
		March 6, 1942	Recovery		12.8	75.4†					5.89	101.0

* Diodrast Tm of nearest date of same period used.

† Creatinine clearances observed during *diodrast* Tm periods.

Columns 6, 7, 8, and 9 are corrected to 1 sq.m. surface area.

TABLE 1—*Concluded*

DOG	SURFACE AREA	DATE	PERIOD DURING WHICH OBSERVATIONS WERE MADE	TESTOSTERONE PROPIONATE TO DATE	DIODRAST Tm	PLASMA CLEARANCES		EFFECTIVE BLOOD FLOW	FILTRATION FRACTION C_{Cr}/C_D	C_D/Tm_D	C_{Cr}/Tm_D	AVERAGE TEMPERATURE DURING Tm_D DETERMINATIONS
						Creatinine	Diodrast					
	sq.m.			mgm.	mgm. iodine per minute	cc. per minute	cc. per minute	cc. per minute	per cent			°F.
S 3	0.54	January 19, 1942	Control		13.3	56.6†					4.26	
		January 26, 1942	Control			45.8†					3.20	
		January 30, 1942	Control		14.3	53.0†					3.70	102.2
		February 3, 1942	Testosterone propionate 100 mgm. daily									
		February 4, 1942	Testosterone	100	18.6	51.4†					2.76	102.4
		February 5, 1942	Testosterone	200	20.2	52.6†					2.60	102.6
		February 6, 1942	Testosterone	300	30.6	40.6†					1.33	102.0
		February 7, 1942	Testosterone	400	30.3	47.0†					1.55	102.0
		February 9, 1942	Testosterone	600	31.3	46.0†					1.47	102.0
		February 18, 1942	Recovery		26.3	46.5†					1.77	101.6
		March 2, 1942	Recovery		16.4	46.7†					2.84	101.0
		March 13, 1942	Recovery		15.1	46.0†					3.05	101.4

Glomerular filtration rate. The creatinine clearance was unaffected by testosterone propionate although, as is usually true of this test, variations from day to day are evident. The variation during the two periods of therapy is no greater than during the control periods.

Effective renal blood flow. No immediate effect on the diodrast clearance followed the administration of testosterone. The diodrast clearance in dog T2 was fairly constant throughout, while in dog F1, observed over a longer period, the clearance varied over a wide range. It is of possible interest that all of the diodrast clearances in this dog, observed after the end of the first series of injections, were lower than those observed before testosterone was given. The clearance in the two injection periods did not, however, differ significantly from the value observed in the preceding control periods.

Diodrast Tm. The outstanding effect of testosterone on the kidney was the striking increase which invariably took place in diodrast Tm (fig. 1). The fall in C_{cr}/Tm_D and in C_D/Tm_D ratios result solely from the increase in the diodrast Tm.

Dog F1. The control period in dog F1 was complicated by the appearance of estrus. A single high level of diodrast Tm was observed at this time, which may have been associated with intrinsic hormonal changes. Two control observations made later were at a lower level. The highest values of diodrast Tm reached during testosterone administration were but little above the single high figure obtained during estrus, but were 44 per cent higher than the two control figures found immediately before the injections were begun. After a three

months' rest period, the original low control levels had not been reached. A second series of injections now produced a further increase in diodrast Tm, which reached 41 per cent above the levels immediately preceding the second test period and 88 per cent above the original lower control figure.

Dog T2. The response of the diodrast Tm in this dog was still greater, for the highest figures obtained were more than double the control.

Dog S3. In the third dog it was decided to follow the rise in diodrast Tm from the beginning of hormone treatment. Accordingly, diodrast Tm was measured daily, beginning twenty-four hours after the first injection. Twenty-four hours after a single dose of 100 mgm. it had risen 34 per cent; after 200
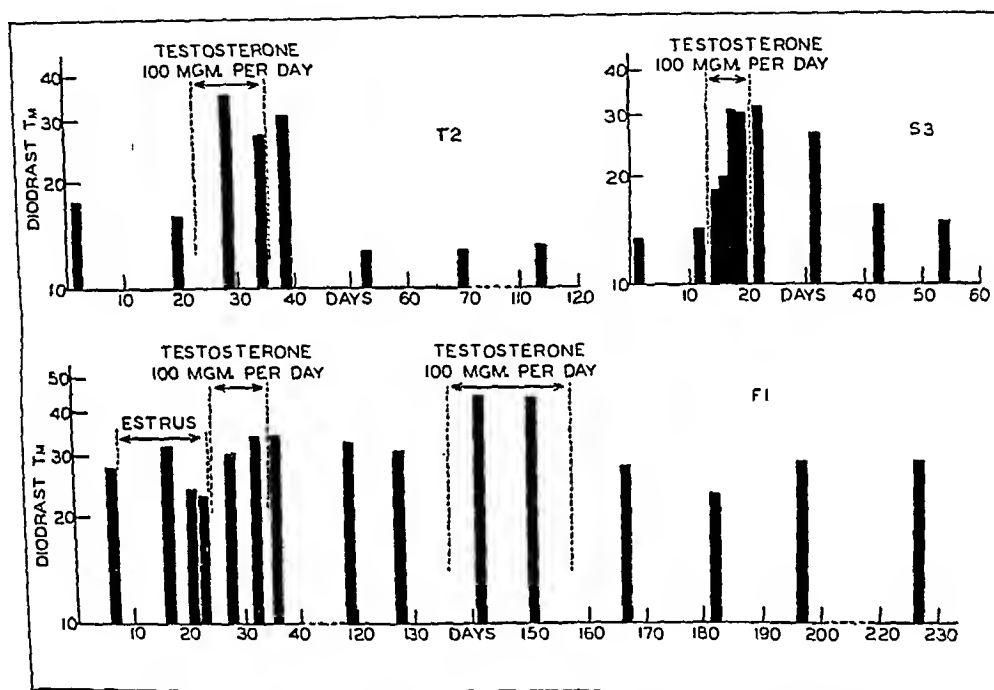
Fig. 1. Diodrast Tm in control periods, during testosterone administration and in recovery periods.

mgm. it had risen 46 per cent; after 300 mgm. the diodrast Tm was more than twice the control value. Beyond this point no increase occurred, for after 400 mgm. and 600 mgm., respectively, the value was close to that found after 300 mgm. Apparently the maximum increase in diodrast Tm had occurred after 300 mgm. had been given, or seventy-two hours after the first dose.

Rate of recovery. The fall in diodrast Tm after suspension of treatment may be rapid, or its final return to normal may take some time. No observations were made early in the recovery period after the first experiment in dog F1. However, three months later her diodrast Tm was almost as high as it had been when she was getting testosterone. The first observation after her second series of injections was made on the tenth day, at which time diodrast Tm was little above the control level. On the third day of the recovery period in dog T2

diodrast Tm had fallen very little but by the seventeenth day it was slightly below the control level. In dog S3, the first observation was made on the tenth day of the recovery period, when diodrast Tm had fallen slightly. Observations on the twenty-second and forty-ninth days after the last injections of testosterone gave values still slightly above the control figures.

~ *Sodium and potassium.* The average daily excretion of sodium and potassium in dog F1 is given by weeks in table 2. It is evident that the output of both sodium and potassium decreases after the administration of testosterone propionate and the increase in diodrast Tm.

The average daily excretion of sodium during the first week of testosterone administration was 0.695 gram, and for the next five days 0.527 gram. These figures are not only lower than the average for the previous seven weeks, which was 0.834 gram, but significantly less than for any single week of the control

TABLE 2

Average daily excretion of sodium and potassium in a seven-week control period and a period of twelve days after testosterone administration

	NUMBER OF DAYS	AVERAGE EXCRETION IN GRAMS OF SODIUM IN 24-HOUR URINE	AVERAGE EXCRETION IN GRAMS OF POTASSIUM IN 24-HOUR URINE	AVERAGE WEIGHT GAIN IN 24 HOURS
				grams
Control.....................	7	0.972	1.068	14
Control.....................	7	0.883	0.982	14
Control.....................	7	0.739	0.737	0
Control.....................	7	0.838	0.788	14
Control.....................	7	0.824	0.792	14
Estrus.....................	7	0.850	0.840	14
Estrus.....................	7	0.730	0.786	14
Testosterone...............	7	0.695	0.622	57
Testosterone...............	5	0.527	0.626	80

period. Similarly, the average daily excretion of potassium for the first week after testosterone was 0.622 gram and for the following five days 0.626 gram, while the average daily amount for the control period was 0.856 gram.

Changes in body weight during the four experiments are given in table 3. There was an average daily increase in weight during treatment which ranged from 0.036 kgm. to 0.083 kgm. The weight gain began from one to three days after the first dose and reached its maximum on the tenth, twentieth, eleventh and fifth days in the four experiments, respectively.

Blood counts done in the control period and toward the end of the testosterone period, did not significantly differ. Neither protein nor abnormal amounts of cellular elements in the urine were ever noted. Blood pressure readings by the indirect palpation method, using a small cuff on the hind leg, were taken at intervals during the control period, while testosterone was being given, and at intervals during the recovery period. Up to four months, three months and two months, respectively, after the administration of these large doses of testo-

sterone, the blood pressure in the three dogs was unchanged. Although several determinations of 17-ketosteroids were made on 24-hour urine collections, no significant increase in the excretion of these substances was notable during the periods of testosterone administration.

DISCUSSION. Testosterone propionate has apparently no effect upon either the glomerular filtration rate or the effective renal blood flow in the dog. The hormone does, however, cause a marked increase in diodrast Tm, revealing an increased functional capacity in the renal tubules to excrete diodrast. In consequence of the increase in diodrast Tm, the relative filtration rate (Ccr/Tm_D) and relative renal plasma flow (C_D/Tm_D) per·unit of functional tubular tissue are reduced. A similar fall in Ccr/Tm_D and C_D/Tm_D has been observed in a woman after unilateral nephrectomy, when a gradual increase in diodrast Tm was not accompanied by similar increase in filtration rate or effective blood flow (unpublished data).

In spite of the decrease in effective renal blood flow per unit of functional tubular tissue to a value as low as one half of the control value, there was no

TABLE 3

Weight gain during testosterone administration

DOG	DAYS	WEIGHT GAIN
		kgm.
F1	12	0.8
F1	21	1.2
T2	11	0.4
S3	6	0.5

elevation in blood pressure. It is possible that the time during which this abnormally low ratio, indicative of relative renal ischemia, existed was too short to produce hypertension. It is by no means certain that the effect of testosterone on the kidney is of benefit. Indeed, increasing diodrast Tm without increasing the blood flow to the tubules might serve only to produce or aggravate renal ischemia. With this point in mind, the injection of testosterone to one of these dogs is being continued for an indefinite period of time.

The increase in the kidney's maximal capacity to excrete diodrast does not necessarily imply an augmentation of other tubular functions. The observed decrease in the excretion of sodium and potassium does, however, suggest the possibility that testosterone administration is followed by a greater reabsorption of these ions as a result of hypertrophied or hyperfunctioning tubules.

The rise in diodrast Tm may be dependent upon an actual hypertrophy of the renal tubules, or it may be based on an increase in function only. Evidence derived from other animals, notably mice and rats, shows that actual tubular hypertrophy does occur after testosterone. However, the maximal increase in mouse kidney weight which has been observed is about 30 per cent, a figure considerably less than the 100 per cent increase in diodrast Tm noted in dogs.

The rapidity of the rise in diodrast Tm does not exclude the possibility of tubular hypertrophy for enlargement of the uterus in response to estrogens occurs as quickly. Changes in the electrolyte pattern of the rat uterus are present within 24 to 48 hours, a fact which has been offered as evidence for the new formation of protoplasm (32). Finally, it must be noted that the proportion of potassium in relation to sodium retained during testosterone administration is too high to permit the assumption that weight gain was simply due to an increase in extracellular fluid. The retained potassium noted here and the nitrogen retention of other reports (10, 11, 12, 13) point strongly to the formation of new tissue, a part of which at least may be represented by tubular hypertrophy.

CONCLUSIONS

1. A rapid rise in the maximal capacity of the kidney to excrete diodrast (diodrast Tm) occurs in the dog following the administration of testosterone propionate. This increase in diodrast Tm may amount to 100 per cent.

2. The change in diodrast Tm is reversible but the time required for original values to be reached varies greatly.

3. The rate of glomerular filtration (creatinine clearance) and effective renal blood flow (diodrast clearance) are unaffected by testosterone propionate.

4. The increase in diodrast Tm is accompanied by a reduction in the urinary excretion of sodium and potassium.

REFERENCES

(1) ALPERT, L. K. Bull. Johns Hopkins Hosp. 68: 522, 1941.
(2) BRODIE, B. B. AND R. STUDINSKI. Personal communication.
(3) BUTLER, A. M. AND E. TUTHILL. J. Biol. Chem. 93: 171, 1931.
(4) CRABTREE, C. E. Endocrinology 29: 197, 1941.
(5) CRABTREE, C. E. Ibid. 29: 204, 1941.
(6) DURLACHER, S. H., D. C. DARROW AND M. C. WINTERNITZ. This Journal 136: 346, 1942.
(7) FOLIN, O. AND H. WU. J. Biol. Chem. 38: 81, 1919.
(8) FUJITA, A. AND D. IWATAKE. Biochem. Ztschr. 242: 43, 1931.
(9) HAWK, P. B. AND O. BERGEIM. Practical physiological chemistry. 11th. ed., Philadelphia, Blakiston, 1937, p. 453.
(10) KENYON, A. T., K. KNOWLTON, I. SANDIFORD, F. C. KOCH AND G. LOTWIN. Endocrinology 26: 26, 1940.
(11) KOCHAKIAN, C. D. Endocrinology 21: 750, 1937.
(12) KOCHAKIAN, C. D. AND J. R. MURLIN. J. Nutrition 10: 437, 1935.
(13) KOCHAKIAN, C. D. AND J. R. MURLIN. This Journal 117: 642, 1936.
(14) KORENCHEVSKY, V. AND M. DENNISON. Biochem. J. 28: 1474, 1934.
(15) KORENCHEVSKY, V., M. DENNISON AND I. BROVSIN. Biochem. J. 30: 558, 1936.
(16) KORENCHEVSKY, V., M. DENNISON AND A. KOHN-SPEYER. Biochem. J. 27: 557, 1933.
(17) KORENCHEVSKY, V., M. DENNISON AND A. KOHN-SPEYER. Ibid., 1506.
(18) KORENCHEVSKY, V., K. HALL AND R. BURBANK. Biochem. J. 33: 372, 1939.
(19) KORENCHEVSKY, V. AND M. A. ROSS. Brit. Med. J. 1: 645, 1940.
(20) LUDDEN, J. B., E. KRUEGER AND I. S. WRIGHT. Endocrinology 28: 619, 1941.
(21) MACKAY, E. M. Proc. Soc. Exper. Biol. and Med. 45: 216, 1940.
(22) McQUEEN-WILLIAMS, M. AND K. W. THOMPSON. Yale J. Biol. and Med. 12: 531, 1940.

(23) PFEIFFER, C. A., V. M. EMMEL AND W. U. GARDNER. Yale J. Biol. and Med. **12**: 493, 1940.
(24) SELYE, H. J. Urol. **42**: 637, 1939.
(25) SELYE, H. Canad. M. A. J. **42**: 113, 1940.
(26) SELYE, H. J. Pharmacol. and Exper. Therap. **68**: 454, 1940.
(27) SELYE, H. AND S. M. FRIEDMAN. Endocrinology **29**: 80, 1941.
(28) SHOHL, A. T. AND N. B. BENNETT. J. Biol. Chem. **78**: 643, 1928.
(29) SMITH, H. W. The physiology of the kidney. New York, Oxford Univ. Press, 1937.
(30) SMITH, H. W., W. GOLDRING AND H. CHASIS. J. Clin. Investigation **17**: 263, 1938.
(31) SMITH, W. W. AND H. W. SMITH. J. Biol. Chem. **124**: 107, 1938.
(32) TALBOT, N. B., O. H. LOWRY AND E. B. ASTWOOD. J. Biol. Chem. **132**: 1, 1940.
(33) TENERY, R. M. AND C. E. ANDERSON. J. Biol. Chem. **135**: 659, 1940.
(34) THORN, G. W. AND L. L. ENGEL. J. Exper. Med. **68**: 299, 1938.
(35) WHITE, H. L., P. HEINBECKER AND D. ROLF. Proc. Soc. Exper. Biol. and Med. **46**: 44, 1940.
(36) WHITE, H. L., P. HEINBECKER AND D. ROLF. This Journal **133**: P489, 1941.
(37) WINTERNITZ, M. C. AND L. L. WATERS. Yale J. Biol. and Med. **12**: 705, 1940.

FACTORS WHICH INFLUENCE THE ACTIVITY OF PURIFIED THROMBIN[1]

WALTER H. SEEGERS AND H. P. SMITH

From the Department of Pathology, College of Medicine, State University of Iowa, Iowa City

It is known that the action of thrombin on fibrinogen is affected by temperature, pH, colloids, and by the presence of certain electrolytes. There has never been any correlated study of these variables. Furthermore, the older studies of isolated variables were carried out with crude mixtures, weak in thrombin and heavily contaminated with antithrombin. We have been able to avoid the latter obstacle by the use of fibrinogen and thrombin which were carefully purified and highly reactive. In addition, identical preparations were used to test each of the variables listed above, thus making it possible to judge the relative importance of each. We propose to discuss the theoretical implications of these findings, and also to point out their application to various technics for the assay of thrombin.

Figure 1, curve *1*, shows the clotting time of standardized fibrinogen solution as related to thrombin concentration. The amount of thrombin required to clot 1 cc. in 15 seconds at 28°C. is defined as a unit of activity. The solution gives clotting times identical to those obtained with the solution required in our 2-stage prothrombin method (1, 2); consequently, when prothrombin conversion is complete a unit of prothrombin is equivalent to a unit of thrombin.

The curve obtained (curve *1*) resembles a rectangular hyperbola $\left(T = \frac{K}{t} \right)$, thus implying inverse proportionality between thrombin concentration, T, and clotting time, t. This "law" has been cited by many of the older workers, and more recently by Quick (3). Calculations show, however, that the experimental points deviate considerably, with the use of the best fitting value of the constant, K. It is only in the middle segment that the correspondence is satisfactory.

It is agreed that the calcium ion plays an important rôle in the formation of thrombin, but there is no agreement regarding its importance in the second phase of clotting. Many have insisted that it does not affect the clotting of fibrinogen by thrombin, but others maintain that complete removal of the ionizable calcium destroys the thrombin action altogether. Our own experience lies between these two extremes. Figure 1, curve *2*, represents results obtained when calcium was omitted, and oxalate was added instead. The clotting times are obviously much longer. To produce a clot in 15 seconds, almost twice as much thrombin is now needed. At other comparable clotting times, the discrepancy between the two curves is equally great. In attempting to remove the

calcium by other means, we have resorted to prolonged dialysis, electrodialysis, and to the use of larger amounts of oxalate. Our experience has been that
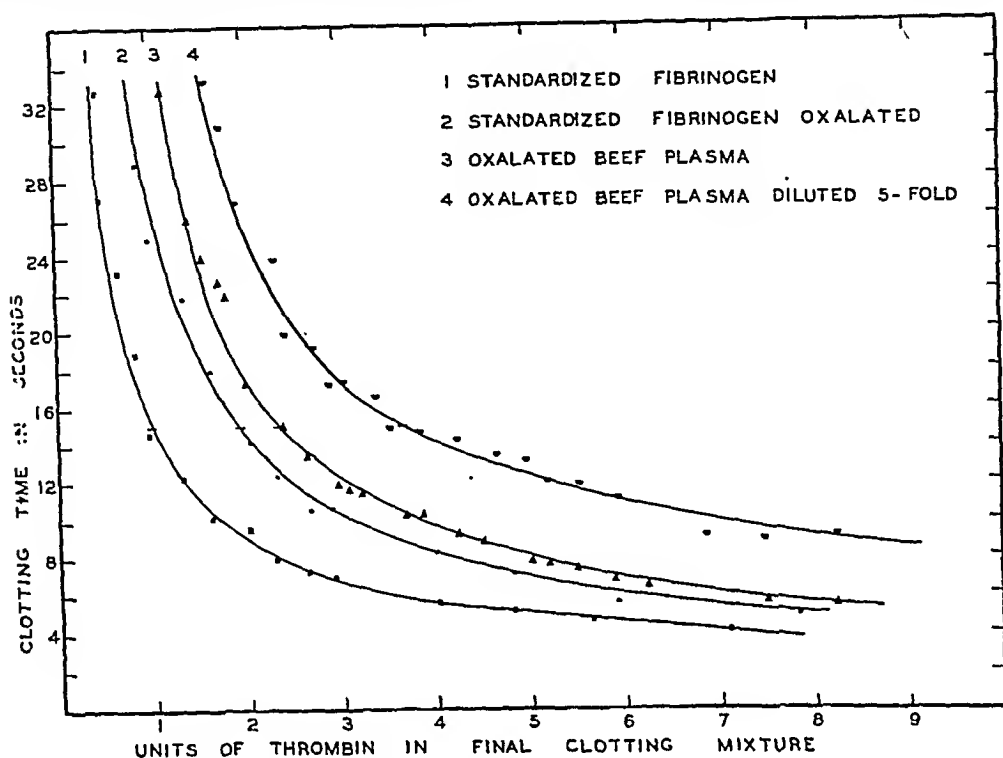
Fig. 1. Clotting time and thrombin concentration. Curve *1*. *a*. Fibrinogen prepared in 5°C.-room. To 900 cc. oxalated plasma (see below) add 90 cc. Mg(OH)$_2$ suspension (2). Centrifuge. This removes prothrombin. To the supernatant plasma, add 240 cc. cold saturated (NH$_4$)$_2$SO$_4$, fortified with 0.3 per cent K$_2$C$_2$O$_4$·H$_2$O. Centrifuge and dissolve precipitate in 900 cc. oxalated saline (0.885 per cent NaCl + 0.092 per cent K$_2$C$_2$O$_4$·H$_2$O), add cold saturated (NH$_4$)$_2$SO$_4$ solution slowly. The first floccules which appear contain much denatured protein. These are eliminated in the centrifuge. To supernatant fluid continue adding (NH$_4$)$_2$SO$_4$ until a total of 315 cc. have been added. Centrifuge, dissolve precipitate in 90 cc. oxalated saline and dialyze against latter for 3 hours at 5°C.

b. Acacia. To remove calcium add an excess of K$_2$C$_2$O$_4$ to a 5 per cent solution of commercial acacia and allow to stand over night. Heat and centrifuge while hot. Dialyze to remove excess oxalate. The acacia is precipitated and dried with alcohol. A trace of salt facilitates precipitation. Prepare a 15 per cent solution in 0.9 per cent NaCl.

c. The clotting mixture consists of 0.335 cc. saline (0.9 per cent NaCl) + 0.066 cc. 1.0 per cent CaCl$_2$ + 0.133 cc. acacia + 0.666 cc. imidazole (7) solution + 0.20 cc. fibrinogen + 0.20 cc. thrombin solution of variable strength (in 0.9 per cent NaCl). From the curve and from the dilution factors, the unit strength of the thrombin is calculated.

Curve 2 is identical to curve 1 except that 0.134 cc. of saline and all of the calcium solution was omitted. In their place 0.20 cc. K$_2$C$_2$O$_4$ (1.85 per cent) was added.

Curve 3. Mix 7 vol. bovine blood with 1 vol. K$_2$C$_2$O$_4$·H$_2$O (1.85 per cent). Centrifuge. To 0.9 cc. oxalated plasma add 0.1 cc. thrombin of variable strength (in 0.9 per cent NaCl).

Curve 4. Same as curve 3, except that the oxalated plasma was first diluted 5-fold with oxalated saline (see above).

sometimes the loss of activity is somewhat greater, but often the loss is less. Complete inactivation never results from any of these procedures.

The mechanism by which the calcium augments the activity of thrombin is still obscure. In addition to calcium ion, it is possible that calcium, in non-ionized form, is an integral part of the thrombin molecule. Analyses for calcium can be considered significant only if the preparation is pure, or nearly so. Published analyses for calcium are based upon thrombin which is less than 5 per cent as potent as ours, which itself is not pure (4). It is thus evident that no no one can state with certainty whether or not pure thrombin would contain bound calcium.

When thrombin is added to oxalated plasma of the same species (fig. 1, curve 3) the clotting time is definitely slower than when a standard mixture of purified fibrinogen is used; in fact, 2.25 units of thrombin were required to produce a clot in the standard 15-second interval. The sluggish clotting is due only in part to oxalation (see curve 2). No doubt the antithrombin of the plasma is also, in part, responsible. In an effort to reduce the effectiveness of the antithrombin, the plasma was subjected to 4-fold dilution with oxalated saline as a preliminary measure. This corresponds very closely to the "dioxalated" test plasma employed so extensively by Bordet (5) and his followers. The result of dilution was disappointing, however (curve 4). The diluted plasma clotted even more slowly than the undiluted plasma. Certain implications of this will be discussed later.

Figures 2A to 2D show the effect of several variables upon the activity of thrombin. In each figure an arrow is used to indicate the level specified as standard in defining the thrombin unit. These chosen levels are the ones at which clotting occurs at the standard 15-second interval. At other points on the curves larger or smaller amounts of thrombin were required to duplicate this 15-second end-point. These variable thrombin values, shown in the charts, indicate the extent to which thrombic activity is affected by deviation from the standard condition.

In the case of temperature, the standard 15-second interval is obtained at 28°C. At 5°C. retardation is quite marked; the amount of thrombin required to cause a clot in 15 seconds is six times as great as at 28°C.

At high temperatures, the clotting efficiency levels off at about 50°C. Beyond this point denaturation occurs even during the brief interval required for assay. Results published elsewhere (6) show denaturation at temperatures as low as 40°C. In the case of many enzymes, the period of assay is quite long and denaturation is therefore conspicuous in the region of 40°C. This offsets the improved catalysis, leading to a so-called "optimum" at this temperature. In the case of thrombin the period of assay is quite brief, but at about 50°C. both thrombin and fibrinogen show denaturation in the brief period of assay, and the clotting efficiency suffers correspondingly.

Neutral electrolytes exert effect upon the conversion of prothrombin into thrombin, and upon the reaction between the latter and fibrinogen. When the electrolyte level is reduced to about 50 per cent of the normal physiological level, there is a decided increase in the clotting power of thrombin (fig. 2B). On the other hand, when the concentration of NaCl is increased from 0.9 per

cent to the level of 1.5 per cent, the activity of the thrombin is reduced by approximately 50 per cent, and at the level of 2.5 per cent NaCl, 5 units of thrombin are required to produce the effect normally given by 1 unit. In making studies on blood coagulation, the problem of neutral electrolytes is commonly ignored. We suggest that clotting mixtures be kept isotonic with respect to 0.9 per cent NaCl. Specific effects can then be assigned to each component in the mixture.

The effect of pH upon the second phase of clotting is shown in figure 2C. We have selected the pH level of 7.2 to 7.5 as standard in the definition of the thrombin unit. To regulate the pH at this level, imidazole buffer is suitable
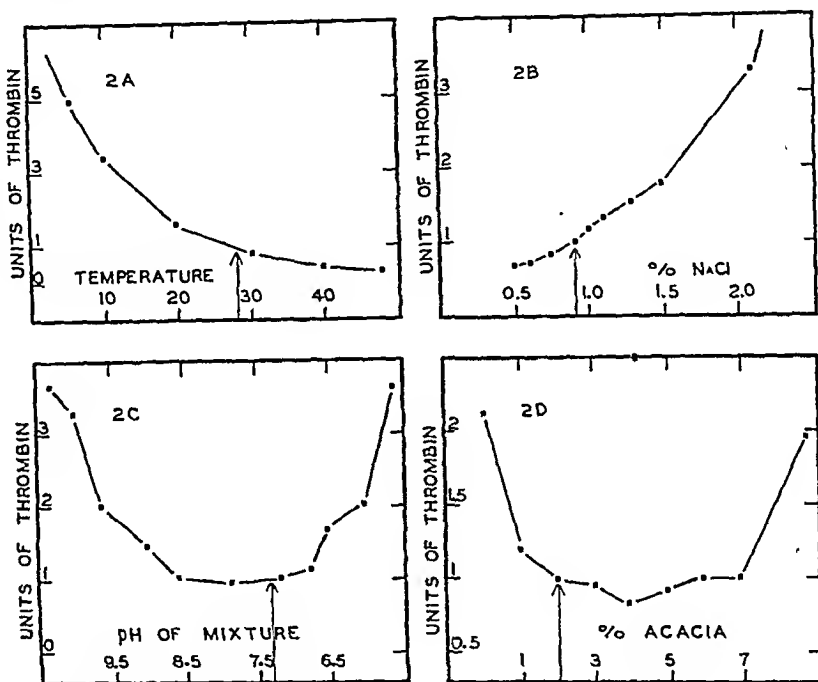
Fig. 2. The effects of temperature, NaCl, pH and acacia on thrombic activity. The composition of the clotting mixtures was analogous to that of curve *1*, figure 1. All values refer to the final clotting mixtures. Those given on the ordinates refer to the amount of thrombin required to form clots in the standard 15-second interval.

(7), for it does not bind calcium or interfere in any known way with the clotting reaction. Figure 2C shows that regulation of pH need not be especially precise, for optimum speed of clotting is observed throughout the entire zone of pH 6.8 to 8.5. Beyond these two points, clotting is markedly impaired, and denaturation of thrombin is observed beyond pH 4.5 and pH 10.

Early in the work of this laboratory it was found that fibrinogen preparations appeared to lose much of their clotting reactivity in the course of preparation. At first, this was attributed to denaturation. It was found, however, that the reactivity could be restored and stabilized by adding any one of a variety of colloids. Acacia proved to be highly effective. The quantitative aspects of this problem have not been recorded heretofore, but are now presented in

figure 2D. The inclusion of 2 to 6 per cent of acacia in the clotting mixture increases the sensitivity at least 4-fold. Larger amounts, however, depress the reaction rate.

In the case of whole plasma, the colloid content is almost optimal for the clotting reaction. The addition of acacia to oxalated beef plasma increases its sensitivity to thrombin only moderately. When oxalated plasma is diluted with oxalated saline, the reactivity is markedly decreased as noted already (fig. 1, curve 3). We believe that this loss of sensitivity is associated mainly with dilution of the colloids, for the addition of acacia to the extent of 2 per cent restores the clotting speed almost completely.

In the assay of thrombin, many of the earlier workers employed oxalated plasma instead of purified fibrinogen. As a rule, the results were expressed in terms of the variable clotting time. Warner et al. (1), using purified fibrinogen, were the first to propose that assay be based upon a fixed clotting time, thus providing a definite thrombin unit. More recently, Astrup and Darling (8) adopted the unit basis of expression. Their unit is the amount of thrombin required to clot 1 cc. of oxalated plasma in 30 seconds. Ice cooled oxalated plasma is mixed with thrombin and "the tube is placed quickly in a water bath at 37°C. and the clotting time is determined." It is obvious from this that the effective temperature is at some indeterminate point between 0 and 37°C. This variability is unfortunate since, as we have shown, the temperature gradient is quite steep between these two points. If one could assume the measurement to be essentially at 37°C., their unit would be smaller than ours on account of temperature (37 vs. 28°C.). On the other hand beef plasma requires 1.3 of our units (fig. 1) for clotting in 30 seconds and this tends to make their unit the larger one. These two variables neutralize each other almost exactly and we conclude that a 37°C. oxalated plasma unit is almost identical to our 28°C. fibrinogen unit. In attempting to duplicate their experiments with "ice cooled" plasma, we found their variable temperature unit to be 40 to 50 per cent larger than ours.

In the assay of thrombin—in fact in all work on blood clotting—one must recognize the fact that new and complex problems may arise from the admixture of clotting factors of different species. This problem has never been studied with purified preparations, but the following experiments will illustrate the general nature of the problem as it pertains to thrombin.

When 2 units of thrombin (0.1 cc.), prepared from bovine plasma, were mixed with oxalated human plasma (0.9 cc.) at 28°C., a clot formed in 15 seconds (table 1). When the same thrombin preparation was mixed with oxalated bovine plasma, or with plasma from the rabbit, approximately 2.2 to 2.5 units were required to form a clot in the 15-second interval. Far more remarkable was the fact that 5 units were required to clot canine plasma at this rate. In the cases of plasma from the pig, 8 units were required, and when plasma of the rat was used, 10 units were needed. In the case of chick plasma, even more thrombin was required. There is no reason to believe, however, that the various types of

fibrinogen are fundamentally different in their reaction to bovine thrombin. In fact, fibrinogen, prepared in purified form from the plasma of the pig reacted as promptly to bovine thrombin as did the purified fibrinogen of bovine origin. It seems clear that other components of the various plasmas have a marked effect in determining the speed with which the fibrinogen and the thrombin interact. In conformity with this view is an observation that 10 units of thrombin clotted bovine plasma in about 5 seconds, even when the latter was diluted with equal parts of saline. However, when this same bovine plasma was diluted with equal parts of chick plasma, 70 seconds were needed for clotting. It is evident that the chick plasma exerted a strong specific inhibitory effect in preventing bovine thrombin from clotting the bovine fibrinogen which was present in the mixture.

In seeking an explanation for these examples of inhibition, we suspected at first that the antithrombin content of plasma might differ markedly from one species to another, but preliminary studies made by incubating the various

TABLE 1

Units of purified beef thrombin required to clot 1 cc. oxalated plasma in 15 seconds at 28°C.

SPECIES	UNITS
Human	2.0
Rabbit	2.2
Beef	2.2–2.5
Canine	5.0
Pig	8.0
Rat	10.0
Beef + 2.7 per cent acacia	0.95
Pig + 2.7 per cent acacia	1.1

plasmas with bovine thrombin failed to reveal any marked differences in rate of thrombin destruction. We have as yet not tried to study these species differences with thrombin prepared from other species. It is possible that precipitin-like reactions which occur between sera of different species are responsible for interference with the clotting reaction. In connection with the solution of this problem there is an interesting experimental fact. If acacia is added to pig plasma to the extent of 2.7 per cent it is possible to clot 1 cc. of the plasma with only 1.1 units of beef thrombin (table 1). This involves an 8-fold reduction in the amount of thrombin required. If one performs the same experiment with beef plasma, the amount of thrombin required changes from 2.5 to 0.95 units. In other words acacia is of benefit within the same species, and it also tends even more markedly to obliterate the striking difference between species.

In conclusion, we wish to suggest that efforts at the assay of thrombin be accompanied by specific use of unit terminology, and that cross-correlation be made with technics of other workers. In many cases it should be sufficient to obtain data with oxalated plasma of the same species.

SUMMARY

A correlated study has been made of a number of factors which affect the action of thrombin on fibrinogen. Under standardized conditions the clotting time is inversely proportional to thrombin concentration, though the correspondence to a simple formula is far from exact. Curves are given to show the effects of temperature, electrolyte concentration, pH, and colloid content of the clotting mixture. Thrombin prepared from bovine plasma does not clot pig plasma as well as it clots bovine plasma. However, purified fibrinogen of the two species is clotted with equal ease. The inhibition is thus due to inhibitory reactions, not to fundamental incompatibility of thrombin of the one species for the fibrinogen of the other. Suggestions are made regarding the fundamental principles to be followed in the assay of thrombin.

REFERENCES

(1) WARNER, E. D., K. M. BRINKHOUS AND H. P. SMITH. This Journal 114: 667, 1936.
(2) SMITH, H. P., E. D. WARNER AND K. M. BRINKHOUS. J. Exper. Med. 66: 801, 1937.
(3) QUICK, A. J. This Journal 115: 317, 1936.
(4) SEEGERS, W. H. Federation Proc. 1: 184, 1942.
(5) BORDET, J., AND L. DELANGE. Ann. de Inst. Pasteur. 26: 657, 1912.
(6) SEEGERS, W. H. J. Biol. Chem. 136: 103, 1940.
(7) MERTZ, E. T. AND C. A. OWEN. Proc Soc. Exper. Biol. and Med. 43: 204, 1940.
(8) ASTRUP, T. AND S. DARLING. Acta Physiol. Scand. 2: 23, 1941.

A STUDY OF THE EFFECTS OF ISOTONIC SERUM AND SALINE INFUSION FOLLOWING TRAUMA IN DOGS

H. GUTMANN, W. H. OLSON, S. O. LEVINSON AND H. NECHELES

Assisted by W. SCRUGGS

From the Samuel Deutsch Serum Center and the Department of Gastro-Intestinal Research, Michael Reese Hospital, Chicago, Ill.

Blalock (1), Best and Solandt (2), and more recently Mahoney (3) have studied the treatment of traumatic shock in dogs. Blalock (1) infused severely traumatized animals with crystalloid solutions. Best and collaborators (2) found pituitrin followed by an adequate quantity of hypertonic serum an effective form of treatment of shock. In Mahoney's experiments plasma did not restore traumatized animals infused 7 hours after trauma (3).

We were interested to study the effects of rapidly induced trauma to muscle and bones, and to investigate whether the prompt infusion of isotonic serum would prove efficacious in the treatment of this condition. To our knowledge there is no convincing evidence in the literature which answers this question.

METHODS. Normal healthy dogs were starved for twenty-four hours; water was not withheld. The animals were anesthetized with sodium pentobarbital. A cannula was inserted in the carotid artery for sampling and for recording of blood pressure with heparin as anticoagulant, and one femoral vein was cannulated. Circulating time was estimated by the sodium cyanide method (4), plasma volume by the method of repeated intravenous injections of the blue dye T-1824 (5), plasma proteins by the falling drop method of Barbour and Hamilton (6), and plasma CO_2 of arterial blood by the Van Slyke and Neill manometric technique. After a suitable control period, during which all determinations were performed, trauma was applied to one lower extremity. A critical condition of the animal was assumed to exist when blood pressure had dropped to 50 mm. Hg and remained at that level for 10 minutes or longer. After trauma, determinations were repeated at stated intervals.

We are not stating that our animals were in a sufficiently grave degree of shock to satisfy every worker in this field (7). However, all our untreated control animals died rapidly in what seemed to be terminally an irreversible condition of shock. Prior to this, their condition was critical enough for the purpose of testing the value of restorative fluids and, by carefully following the physical and chemical determinations before and after infusions, certain definite conclusions could be drawn. If we were to accept the extreme opinion that shock must be an irreversible state leading to death (7), all work on the therapy of shock would be useless.

We have not been able to standardize the amount of trauma administered. This conforms with the observations of others (7). The size of the animal, strength and position of different structures, and individual resistance seem to

make standardization impossible.   We employed a constant weight dropped on the middle of the left thigh from constant height at constant intervals.   The number of blows necessary to reduce blood pressure to 50–60 mm. Hg in a group of 15 dogs of approximately the same weights varied as follows: 92, 116, 142, 166, 167, 178, 179, 205, 246, 355, 389, 429, 431, 530 and 987.   We therefore resorted to administering a number of blows sufficient to reduce blood pressure to 50 mm. for a period of 10 minutes or longer, thus accepting low pressure as a preliminary indication of a serious condition of the animal and later correlating this with all the other more time-consuming determinations performed.

For the determination of the concentration of T-1824 in the arterial blood the spectrophotometer was used, employing a wave length of 620 millimicrons. The log of transmission was directly proportional to the concentration of the dye in plasma within a range of 10 to 60 mgm. per cent in the plasma T-1824.   Following the injection of the dye its concentration in the arterial blood of the normal dog was constant after 4 minutes, and remained so for two hours and longer. In the traumatized animal with a blood pressure of 50 mm. and a circulating time of 41 seconds, mixing of the dye was complete after five minutes, and only 6.4 per cent of the dye had disappeared within eight minutes following injection, at which time the blood pressure and the circulating time had not changed.   From a number of such experiments we felt that we were able to rely on the average of two determinations of the concentration of T-1824 in the plasma, five and eight minutes after the injections, respectively.   In the traumatized animal the error of plasma volume determination was greater than in the non-traumatized animal, but we feel that determinations were still within the limits of error of the method and of biological variations.   In order to control whether blood from animals in shock itself would have an effect on the optical properties of the plasma, blood was obtained from dogs before and after severe trauma, and mixed with suitable amounts of T-1824.   Since absorption proved to be identical in both cases, we concluded that the blood of dogs in severe shock does not itself alter the accuracy of the determination of T-1824.

Care must be exercised in the preparation of dog's serum or plasma if severe serum reactions are to be avoided.   Employing the procedure of Goodner and collaborators (8), we have been able to reduce the instances of plasma and serum reactions to a minimum.   The only disadvantage of the method seems to be that about 1 per cent of proteins are lost.   The use of rubber and glassware free from pyrogenic material, i.e., carefully cleaned with alkali and water and sterilized, seems to be essential.   No typing was necessary, since we have found that in the dog hemagglutinins are not present in serum (9).

RESULTS.   A. *Saline and serum infusion in the same animal.* (Fig. 1.)   In order to eliminate the variable response of different animals, double infusion experiments were performed on the same dog (4 expts.).   Figure 1 represents a typical experiment.   It demonstrates graphically the transient effects of saline and the more lasting and beneficial effects of serum infusion.

In other experiments the second infusion was with glucose saline solution in-

stead of serum. These animals died shortly after the saline infusion, with blood pressures falling rapidly after completion of the infusion. The first and particularly these latter experiments confirmed the results of Blalock (1) and others, that saline infusion may even be harmful under certain conditions.

B. In the following series of experiments a more extensive study of the changes in the animals following trauma was performed, and the results are summarized in table 1. Following trauma and a low blood pressure maintained at 50, as described under Methods, serum or saline solution were administered intravenously, 40 cc. per minute. Table 1 represents the averages of three groups of determinations demonstrating the changes following trauma and the effects of serum and
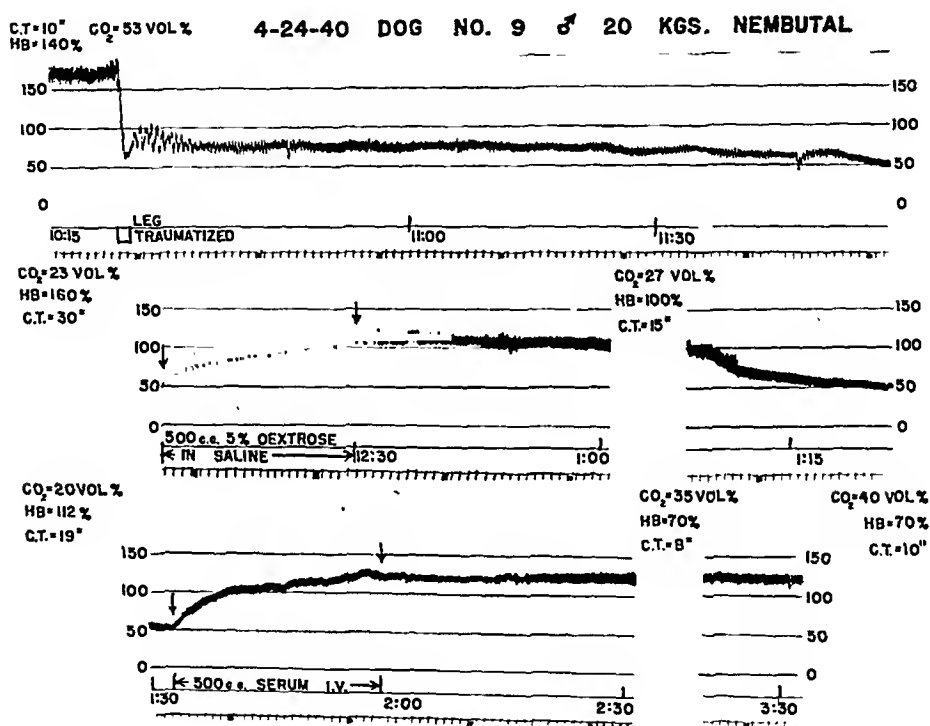
Fig. 1. Trauma. Effect of infusions with saline-glucose solution and with serum

saline respectively upon the course of developing shock.[1] The last determination was done at the end of the experiments; thus, in the serum group in which all animals lived longer than $4\frac{1}{2}$ hours, at the time when the experiment was stopped, and in the saline group, when blood pressures had dropped to 40–50 mm. Hg, i.e., some time before the death of the animal.

The average survival time for the serum group was 405 minutes. Only one dog of the saline group lived for 270 minutes, while the other six dogs died after 145

[1] More determinations were performed, but only 3 are given in the table, representing the most important points.

minutes. It is significant that 145 minutes after trauma the plasma volume of the saline dogs was equal to the plasma volume of the serum dogs 405 minutes after trauma. In order clearly to illustrate this difference, the individual values for plasma volumes of each group were plotted against time. The values for plasma volume "after infusion" were calculated by adding the infused volume of saline and serum respectively to the plasma volumes "after trauma." This was done because the rapid changes following infusion make plasma volume determinations at that time to appear of doubtful value. From this graph values for plasma volumes were averaged for half-hourly periods. Chart 1 represents these

TABLE 1

Averages of determinations in dogs before and after trauma and transfusion with serum or saline

6 DOGS. 25 CC. PER KGM. SERUM	CONTROL	IMMEDIATELY AFTER TRAUMA	SURVIVALS. 6 DOGS, 405 MINUTES	DEATHS
Blood pressure (mm. Hg)	164	72	126	
Circulating time (seconds)........................	9.7	23.3	17	
Plasma volume (ec. per kgm.)....................	40.5	25.0	34.6	
Plasma proteins (gr. per cent)....................	6.75	6.60	6.95	
Plasma proteins, total eirculating (gr. per kgm.) .	2.73	1.65	2.40	
Hematocrit (per cent)............................	52.7	55.1	43.6	
Plasma CO$_2$ (volume per eent)..................	48.2	34.1	31.2	

7 DOGS. 25 CC. PER KGM. SALINE			SURVIVALS. 1 DOG, 270 MINUTES	DEATHS. 6 DOGS, 145 MINUTES
Blood pressure (mm. Hg)	172	57	60	43
Cireulating time (seconds)........................	9.8	24.5	47*	24.8
Plasma volume (cc. per kgm.)....................	46.2	33.7	31.8	33.4
Plasma proteins (gr. per cent)....................	6.31	6.06	5.54	4.94
Plasma proteins, total circulating (gr. per kgm.).	2.92	2.02	1.76	1.65
Hematocrit (per cent)............................	49.0	50.1	49.5	42.7
Plasma CO$_2$ (volume per cent).............	50.6	29.0		19.0

* Shortly before death.

results expressed as per cent changes from the control plasma volumes before trauma.

Following severe trauma a loss of 29 to 37 per cent of the available circulating plasma occurred in 11 dogs. Following the infusion of serum, plasma volume was elevated above the control value and slowly dropped to and slightly below it within 3 hours. This shows that adequate, i.e., large serum infusions are a safe method for elevating and maintaining a more or less normal plasma volume following severe trauma.

It is apparent that one hour after infusion of saline a quantity of fluid equal to 98 per cent of the infused fluid had left the effective circulation. This volume of fluid lost is not saline, but saline-diluted plasma. This is apparent from the

plasma protein determinations of two typical experiments, represented in chart 2[2].

Chart 2 demonstrates that in the case of saline infusion, the plasma proteins were not only greatly diluted, but also that the capillary walls became more permeable and more plasma left the circulation ("washing out" effect of Blalock (1)). In the case of serum infusion, the level of plasma protein was raised above the control value and remained there more or less until the experiment was stopped (the animal survived). The values for plasma CO_2 on chart 2 demonstrate the evanescent and possibly harmful effects of saline infusion, and emphasize the property of serum (or plasma) to restore and maintain a more or less normal plasma CO_2 level and thus counteract acidosis.

DISCUSSION. Shock produced by trauma may be relieved by the intravenous administration of serum even when shock is severe, but only when administered without much delay and in sufficient amounts. Low blood pressure, low circula-
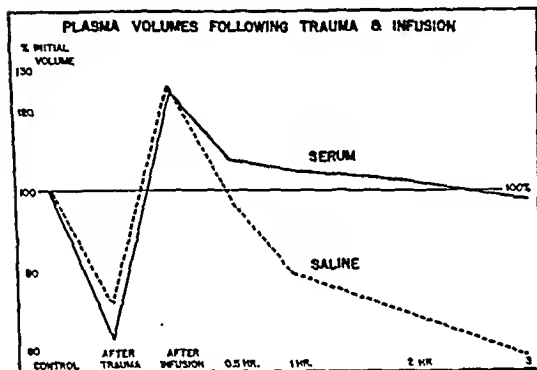
Chart 1
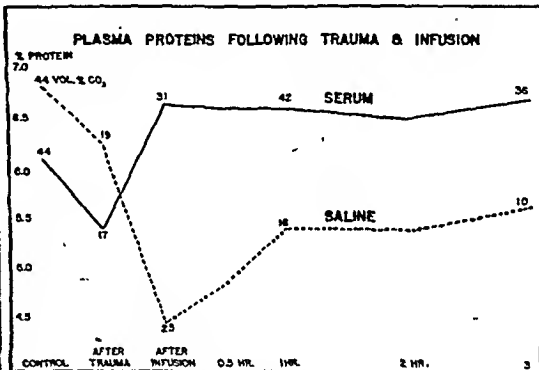
Chart 2

Chart 1. Trauma. Averages of plasma volumes expressed in per cent changes from controls before trauma. The value "after infusion" is calculated (see text).

Chart 2. Trauma. Plasma protein values of 2 representative experiments. The numbers above or below the curves represent plasma CO_2 (vol. per cent).

tion rate, low plasma CO_2, hemoconcentration, and low plasma proteins can be restored to approximately normal values; plasma volume may be restored to normal or above and held at that level for a number of hours, after which it slowly declines. The reverse seems to be true in the case of crystalloid solutions. Plasma volume is raised, but only over a short period of time. Following serum infusion some of the infused proteins are lost, but most evidently are retained in the circulation. On the other hand, following infusion with saline, all or most of the saline administered is lost, together with circulating plasma proteins. Thus saline infusion may be definitely harmful in traumatic shock.

[2] It is not possible to average the per cent values of plasma proteins of all dogs in each group, and the computation of averages of the total plasma proteins per kgm. weight of dog seemed to involve serious mistakes because these average values did not agree with the values of the individual experiments. Therefore, the results of two representative experiments are reproduced here.

Serum administered after the failure of saline to improve the condition of the dog in traumatic shock (three-quarters of an hour after the saline solution) still can restore blood pressure, plasma CO_2, and circulating time to values slightly below normal and well compatible with life. A preoperative infusion of a traumatized animal with serum permitted amputation of the leg and the second infusion raised blood pressure, blood CO_2, blood hemoglobin, hematocrit, and circulating time to near control values, well compatible with life. Repeated saline infusions, administered shortly after trauma at blood pressures of 50 to 60, apparently hastened death and did not establish a condition favorable to amputation.

SUMMARY

Anesthetized dogs were traumatized severely and the effects of infusions with saline or serum were studied by a number of physical and chemical determinations.

The effects of saline-glucose infusions were as follows:

1. Five dogs died 145 minutes and one dog died 270 minutes after infusion.

2. A transitory rise and, usually, a gradual fall of blood pressure soon after termination of infusion. Two hours after infusion blood pressure was 43 mm. Hg in 5 dogs.

3. An amount of saline-diluted plasma, equal to or larger than the amount of saline-glucose infused, had left the effective circulatory volume within about one hour after infusion.

4. Plasma proteins were greatly diluted immediately after infusion; after that some hemoconcentration took place, but total plasma proteins never reached control levels again, indicating that proteins were "washed out" of circulation together with the saline-glucose solution.

5. A slight transient improvement of arterial plasma CO_2 shortly after infusion, followed by a continuous and more or less rapid fall to low levels.

6. Circulating time improved for a short period after infusion and then continuously increased toward the end.

The effects of infusion with normal dog's serum were as follows:

1. The dogs lived for 405 minutes, and then the experiments were terminated.

2. A rise of blood pressure to normal or near normal levels, usually maintained till end of experiment.

3. A large part of the infused volume of serum remained in the effective circulation throughout the period of observation.

4. Plasma proteins were always raised to or above control levels and remained there throughout period of observation.

5. Plasma CO_2 was always markedly improved and in a number of experiments continued to rise to higher levels one hour after infusion.

6. Circulating time returned to control levels and then slowly increased towards termination of experiments.

Infusion with serum after saline had failed to restore animal, was followed by marked improvement of animal.

REFERENCES

(1) BLALOCK, A. Principles of surgical care, shock and other problems. Mosby, 1940, p. 158 ff.

(2) BEST, C. H. AND D. Y. SOLANDT. Canad. M. A. J. **43**: 206, 1940; Brit. Med. J. **1**: 799, 1940.

(3) MAHONEY, E. B. Ann. Surg. **108**: 178, 1938.

(4) OLSON, W. H., H. GUTMANN, S. O. LEVINSON AND H. NECHELES. War Medicine **1**: 830, 1941.

(5) GIBSON, J. G. AND K. A. EVELYN. J. Clin. Investigation **17**: 153, 1938.

(6) BARBOUR, H. G. AND W. F. HAMILTON. J. Biol. Chem. **69**: 625, 1926.

(7) WIGGERS, C. J. Physiol. Rev. **22**: 74, 1942.

(8) GOODNER, K., F. L. HORSFALL AND R. J. DUBOS. J. Immunol. **33**: 279, 1937.

(9) OLSON, W. H. This Journal **129**: 433, 1940.

THE NERVOUS FACTOR IN THE CIRCULATORY FAILURE INDUCED IN ADRENALECTOMIZED DOGS BY INTESTINAL STRIPPING AND A SINGLE STAGE BILATERAL ADRENALECTOMY

W. KLEINBERG, J. W. REMINGTON,[1] V. A. DRILL AND W. W. SWINGLE

From the Section of Physiology, Biological Laboratory, Princeton University, Princeton, New Jersey

In a recent study on the ability of various adrenal steroids to protect the adrenalectomized dog against the circulatory failure which follows a 25 minute gentle stripping of the small intestine, trauma to muscle masses of the hind limb, bilateral adrenalectomy at a single stage operation and hemorrhage, it was found that whereas corticosterone and 17-hydroxy-11-dehydrocorticosterone afforded adequate protection after all four of the stress procedures, desoxycorticosterone acetate was seemingly without effect after the single stage bilateral adrenalectomy or the intestinal stripping (1). This lack of protective action was striking in view of the established fact that desoxycorticosterone maintains the adrenalectomized dog in good health and vigor, and can, under certain conditions, protect against the circulatory collapse following the other shock inducing procedures employed (1). It would seem that the single stage bilateral adrenalectomy and the intestinal stripping must have a common factor contributing toward the circulatory failure, against which desoxycorticosterone, unlike corticosterone, 17-hydroxy-11-dehydrocorticosterone or whole gland cortical extract, is ineffectual. At first sight the two procedures seem to have little in common. The one involves but little trauma, with no discernible local loss of fluid (2). Intestinal stripping, on the other hand, involves a widespread and extensive trauma, with significant local fluid loss and resultant hemoconcentration. Blood chemistry changes after both procedures have proved negligible, and offer little evidence for the existence of a shock inducing factor common to both procedures. Only a tendency toward a decline in blood sugar, which may or may not be severe and is by no means invariable, seems common to both (2).

Freud, Uyldert and Waterman (3) pointed out the similarity between certain symptoms which follow adrenalectomy in the dog and those observed after extensive injury to major elements of the sympathetic nervous system in the splachnic area. Stiemens (4) has shown that in the dog the adrenal glands are in close proximity to the main mass of the coeliac plexus itself. Workers in this laboratory have demonstrated that careful infiltration of the area surrounding the adrenal glands with a procaine solution prior to their removal will prevent the appearance of any symptoms of circulatory failure (5). It seems clear, therefore, that trauma to adjacent nervous structures must play a significant rôle in the production of the circulatory collapse which follows the single stage bilateral adrenalectomy in the dog.

[1] Upjohn Research Fellow.

In an effort to define more exactly the nervous elements probably subjected to trauma at the time of the adrenalectomy, careful dissections were made of the adrenal region in a number of dogs. While considerable variation in detail exists between animals, figure 1 may be taken to represent a typical dissection. Dividing the coeliac plexus into its eight major component parts, the two coeliac, two superior mesenteric, two adrenal, and two renal (which may be diffuse) ganglia, it can be seen that the adrenal glands are immediately adjacent to three, and but shortly removed from the other five nerve masses. Numerous fibers ramify in the capsules of the glands, linking them to the two adrenal ganglia and to the left superior mesenteric ganglion. Damage to these ganglia is almost unavoidable during the operation. Further, in some animals the
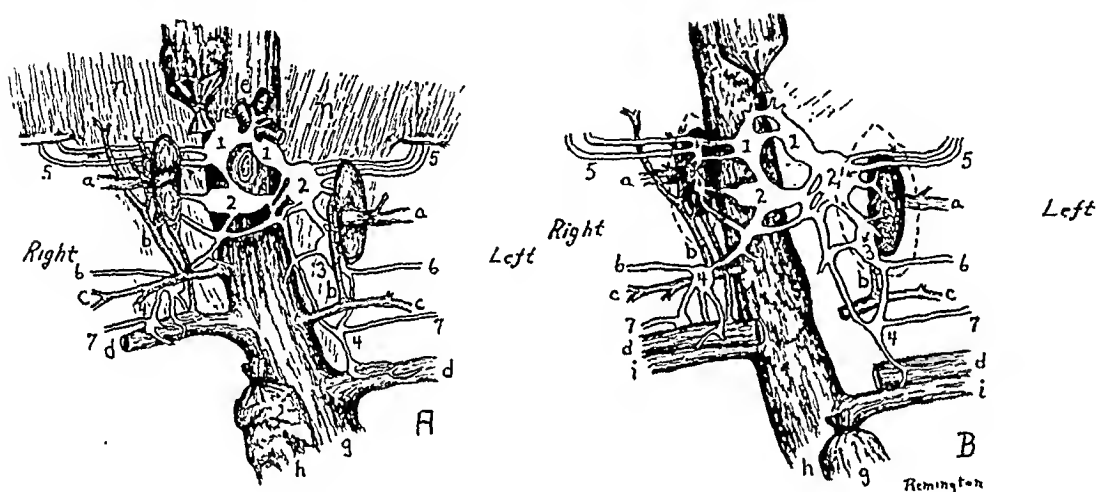
Fig. 1. Surgical anatomy of the adrenal gland of the dog. A. Ventral view, vena cava removed. B. Dorsal view, dorsal aorta removed. Arrows denote points and direction of direct injection with procaine solution. Areas enclosed by dotted lines represent probable minimal area of thorough procaine infiltration. 1, coeliac ganglion; 2, superior mesenteric ganglion; 3, adrenal ganglion; 4, renal ganglion; 5, greater splachnic nerve; 6, lesser splachnic nerve; 7, least splachnic nerve. a, adreno-lumbar vein; b, adrenal artery; c, lumbar artery; d, renal artery; e, coeliac artery; f, superior mesenteric artery; g, dorsal aorta; h, vena cava; i, renal vein.

anatomical association of the glands and the greater splachnic nerves may be such that these nerves also are subject to extensive trauma.

On the basis of these findings, the simple procaine infiltration technique developed for the single stage bilateral adrenalectomy was modified in that the adrenal ganglia, left superior mesenteric ganglion, and greater splachnic nerves are carefully freed from surrounding tissue, and direct injections of a 4 per cent procaine solution made into the nerves and the mass of the ganglia (fig. 1). The close proximity of the right adrenal gland to the vena cava limits the area available for direct procaine injection on this side, but connecting nervous elements between the adrenal ganglion and the right coeliac and right superior mesenteric ganglia can usually be isolated and treated. A generalized bathing and infiltration of the tissues surrounding the adrenal glands is then made, the

glands cleared by blunt dissection, and removed.   A total of 3 to 5 cc. procaine solution is used on each side, the amount varying with the ease of infiltration and the extent of dilution by peritoneal fluid. .

It is our impression that the usual minimal area well anesthetized by such a procaine infiltration is approximately that shown in figure 1.   It is not improbable that this simple procedure is sufficient to completely narcotize the whole coeliac plexus.

Only rarely does an animal subjected to a single stage bilateral adrenalectomy survive longer than 24 hours, with the usual life span of either untreated or desoxycorticosterone acetate treated animals falling between 9 and 14 hours. Since developing the procaine infiltration technique, we have routinely prepared adrenalectomized dogs by the single stage operation.   In a series of over 50 dogs, there has been not a single clear failure.   A typical post-operative recovery is shown in table 1.   These animals usually eat within 6 to 8 hours following the operation, show little if any decline in blood pressure, and can be maintained on small amounts of desoxycorticosterone acetate.[2]

Since the coeliac plexus contains both afferent and efferent fibers of the sympathetic nervous system, as well as preganglionic fibers of the vagus nerve, any or all of three nerve pathways may reasonably be assumed to be involved in the induction of the circulatory failure.   However, if it could be shown that a protection to the circulation comparable to that obtained by the local infiltration with procaine is afforded by either spinal anesthesia or spinal cord section prior to adrenal removal, then the nerve elements concerned could hardly be other than visceral afferent fibers.

Actually, adequate protection is afforded by either of these procedures.   Firor routinely prepares adrenalectomized dogs by a single stage bilateral adrenalectomy under spinal anesthesia, and the animals can be maintained from the time of operation by desoxycorticosterone acetate (6).   We have had similar results with spinal anesthesia (table 1).   The blood pressure of such operated animals remains low during the period of the anesthesia, but returns to normal levels when the anesthesia wears off.

The ability of spinal section to protect against the circulatory failure which rapidly follows a single stage bilateral adrenalectomy is illustrated by dog 1, table 1.   As was to be expected, the blood pressure fell after the spinal section and remained low.   The significant point is that the animal recovered well from the operation, taking food within 10 hours of its completion.

The widespread innervation of the small intestine by sympathetic fibers and visceral afferents renders plausible the suggestion that the initiating factor in the circulatory failure which follows stripping the intestine in the adrenalectomized dog may also be an extensive injury to the nervous elements of the splanchnic area.   Either untreated adrenalectomized dogs, or those given prophylactic foretreatment with desoxycorticosterone, show a progressive decline

in blood pressure after twenty-five minutes of intestinal stripping, with death usually occurring at about the ninth hour although the life span may vary from 6 to 18 hours. Desoxycorticosterone treated dogs subjected to spinal section prior to the intestinal stripping show no such progressive decline in pressure

TABLE 1

Effect of nerve block on the response of the dog to a single stage bilateral adrenalectomy

DATE	TIME	BLOOD PRESSURE	PULSE	BLOOD SUGAR	HEMO-GLOBIN	REMARKS
Dog 1, 12.0 kgm. Spinal section						
		mm. Hg	*per minute*	*mgm. per cent*	*grams per cent*	
1/26	10:30 a.m.	120	100	84	15.0	Spinal cord cut at T-1
1/28	10:30 a.m.	74	140	80	15.1	Both adrenals removed, no anesthesia
	9:00 p.m.	79	140	68	15.2	Alert, ate food. Given 5 mgm. desoxycorticosterone acetate
1/29	10:30 a.m.	78	132	78	15.0	Good condition, experiment discontinued
Dog 2, 10.4 kgm. Procaine infiltration of coeliac area						
2/4	11:00 a.m.	110	80	86	14.6	Both adrenals removed, using local procaine infiltration
	9:00 p.m.	105	120	74	14.6	Good condition, ate food. Given 3 mgm. desoxycorticosterone acetate
2/5	11:00 a.m.	108	84	80	14.7	Normal, experiment discontinued
Dog 3, 10.7 kgm. Spinal anesthesia						
4/22	10:30 a.m.	130	84	92	15.4	Both adrenals removed, using spinal anesthesia
	10:30 p.m.	90	134	81	15.4	Good condition, ate food. Given 3 mgm. desoxycorticosterone acetate
4/23	10:30 a.m.	131	128	96	15.6	Normal, experiment discontinued

from the lowered level attained after section of the spinal cord, and survive well beyond the critical period (table 2).

It has proven difficult to keep the adrenalectomized spinal dog in good condition. Regardless of the recovery interval allowed after the cord section, the removal of the adrenals is followed by a gradual failing of the appetite and general health of the animal. After a few days, respiratory infections often

TABLE 2

Effect of nerve block on the response of the adrenalectomized dog to intestinal stripping

DATE	TIME	BLOOD PRESSURE	PULSE	BLOOD SUGAR	HEMO-GLOBIN	REMARKS
		mm. Hg	*per minute*	*mgm. per cent*	*grams per cent*	
Dog 4, 10.3 kgm. Spinal section. 20 mgm. DCA*					
3/3	10:30 a.m.	120	120	92	14.7	Right adrenal removed, using procaine infiltration. Spinal cord then cut at T-2, left adrenal removed using procaine infiltration. Intestine stripped for 25 minutes
	1:45 p.m.	66	96			Completed stripping
	9:15 p.m.	68	110	76	15.0	
3/4	12:30 p.m.	65	152	79	15.3	Alert, given food and water
3/5	10:00 a.m.	48	170	118†	15.2	Showing symptoms of respiratory infection. Sacrificed on 3/6
	Dog 5, 13.0 kgm. Procaine infiltration of the coeliac area. 20 mgm. DCA*					
3/5	10:00 a.m.	116	123	82	15.1	Bilateral adrenalectomy with procaine infiltration. Intestine stripped for 25 minutes
	1:45 p.m.	99	146			Completed stripping
3/6	10:15 a.m.	98	132	71	18.6	Alert, refused food but took water
3/7	10:00 a.m.	100	130	74	18.2	Appears normal, experiment discontinued
	Dog 6, 10.6 kgm. Procaine infiltration of the coeliac area. 20 mgm. DCA*					
3/6	11:45 a.m.	121	52	71	15.1	Bilateral adrenalectomy with procaine infiltration. Intestine stripped for 25 minutes
	3:30 p.m.	100	180			Completed stripping
	9:30 p.m.	72	240	52	15.6	
3/7	9:30 a.m.	66	162	76	15.6	Sluggish, took food and water
3/8	9:30 a.m.	93	108			Appears normal, experiment discontinued

TABLE 2—*Concluded*

DATE	TIME	BLOOD PRESSURE	PULSE	BLOOD SUGAR	HEMO-GLOBIN	REMARKS
Dog 7, adrenalectomized, cortical extract maintenance therapy, 10.3 kgm. Spinal anesthesia. 20 mgm. DCA*						
		mm. Hg.	*per minute*	*mgm. per cent*	*grams per cent*	
4/18	10:00 a.m.	128	124		11.8	Intestine stripped for 25 minutes
	11:30 a.m.	71	76			Completed stripping, spinal anesthesia maintained for 5 hours
4/19	11:30 a.m.	131	140		14.8	Normal
Dog 8, adrenalectomized, cortical extract maintenance therapy, 10.9 kgm. Spinal anesthesia. 20 mgm. DCA*						
4/27	11:30 a.m.	112	132	84	12.4	Intestine stripped for 25 minutes
	12:30 p.m.	80	140			Completed stripping, spinal anesthesia maintained for 5 hours
	8:00 p.m.	98	192	68	14.0	
4/28	12:30 p.m.	118	140	73	13.7	Normal

* Desoxycorticosterone acetate given in four intramuscular injections of 5 mgm. each at 18, 12 and 2 hours before, and at the time of operation.

† Dog received food several hours before sample.

intervene, requiring the eventual sacrifice of the animal. We feel that this failure can be attributed, in part at least, to an inability of desoxycorticosterone to adequately maintain the spinal adrenalectomized animal. The use of cortical extract for maintenance was obviated because of the protective action it would have had in preventing circulatory failure following the intestinal stripping which was to follow. The minimal maintenance level of cortical extract is apparently increased to an appreciable extent after the cord section. The experimental procedure was therefore modified so that spinal section single stage bilateral adrenalectomy and the intestinal stripping were all performed at a single sitting. Thorough infiltration of the adrenal area with 4 per cent procaine was made at the time of adrenalectomy. Successful prevention of any symptoms of circulatory failure following this drastic operative procedure have been regularly accomplished.

Since this latter technique involves procaine infiltration of the coeliac region as well as spinal section, the question at once arises whether it is not possible to prevent the circulatory collapse which follows intestinal stripping by procaine infiltration of the nerves and ganglia of the splanchnic area alone. The animals cited in table 2 show clearly that this is often possible. Of a series of seven dogs, but four have shown uneventful recoveries and been successfully main-

tained on desoxycorticosterone acetate. Failures following the use of this procedure probably reflect an inadequacy of thorough infiltration; at least, in every case where the operators were convinced at the end of the operation that the procaine infiltration had been complete and adequate, the animals survived after the intestinal stripping. When the infiltration was doubtful, either because of a confined operative area or undue dilution of the procaine by blood oozing from small vessels or seepage of peritoneal fluid, the animals failed to recover.

Spinal anesthesia will also adequately protect the adrenalectomized dog against the circulatory failure following intestinal stripping. Vigorous adrenalectomized dogs receiving a bare maintenance dose of cortical extract were used. Extract was discontinued the day previous to operation and a priming dose of desoxycorticosterone substituted (table 2). Spinal puncture was performed at the level of the fifth lumbar vertebrae and 2.5 cc. of a 2 per cent procaine solution injected. A small dose of nembutal is given intravenously previous to spinal puncture. At intervals varying from one to two hours, for the first five hours following stripping, 1 cc. doses of the 2 per cent novocaine solution are injected into the cord in order to prolong the spinal anesthesia. The needle is left in the spinal canal throughout the entire procedure. Animals so treated show no sign of circulatory failure and after recovery from the anesthetic are active and vigorous.

DISCUSSION

The evidence seems clear that trauma to nervous elements in the splanchnic area is an initiating factor in the induction of circulatory failure which follows both the single stage bilateral adrenalectomy and the intestinal stripping in the adrenalectomized dog. Since a thorough infiltration of the coeliac plexus with procaine will protect the animal against both procedures, one might conclude that the nerve pathways involved are common to both. In as much as spinal section or spinal anesthesia also affords adequate protection against the circulatory failure, the visceral afferent fibers are apparently responsible. It seems not improbable that barrages of noeiceptive stimuli arising in the area of injury will induce extreme excitation of the vasomotor centers, eventuating in intense vasoconstriction of peripheral vessels over the body. There is no reason to suspect an exhaustion of the vasomotor centers, themselves. It is more probable that the passage of these afferent impulses to higher centers, unless prevented by spinal section, spinal anesthesia, or procaine infiltration of the coeliac plexus and major splachnics, would lead to gradual exhaustion of some part of the peripheral vasculature and the consequent development of circulatory failure.

Of the blood chemistry changes associated with the circulatory failure following the two stress procedures under discussion, only the tendency for a decline in blood sugar seems important (2). It is not our belief that the symptoms of circulatory failure can be clearly attributed to a low blood sugar level, but that the blood sugar change is probably reflecting some deep-seated derange-

ment in carbohydrate metabolism. We do not know how much of this tendency toward a lowered blood sugar can be attributed directly to the trauma of the nervous elements of the splanchnic region (cf. 7). A direct correlation seems to exist, however, between the capacity which the various adrenal steroids possess in protecting against circulatory failure and the activity they show in maintaining normal carbohydrate metabolism in the body (1). For example, 17-hydroxy-11-dehydrocorticosterone, which possesses marked carbohydrate activity, adequately protects the adrenalectomized dog against circulatory failure after all stress procedures employed. Assuming that the presence of these carbohydrate active steroids is essential for the maintenance of a normal carbohydrate cycle, and hence normal function in all tissues of the body, then the ability of the vascular periphery to sustain normal function, in the face of the prolonged stimulation to which it would be subjected as a result of the trauma to nervous elements of the splanchnic area, would be reduced in the adrenalectomized dog given only desoxycorticosterone therapy. For desoxycorticosterone, unlike corticosterone, 17-hydroxy-11-dehydrocorticosterone, and whole adrenal cortical extract, shows no carbohydrate activity.

The eventual circulatory failure in the desoxycorticosterone treated adrenalectomized dog could then be abscribed to two major factors. The first is the intense nervous bombardment of the vascular peripheral apparatus via the vasomotor centers resulting directly from extensive trauma to visceral afferent nerves in the splanchnic area. The interruption of the nervous pathway to the higher centers by spinal section, spinal anesthesia, or local procaine anesthesia, will indirectly protect the end organ against exhaustive stimulation and hence prevent the circulatory collapse. The second factor would be the lessened ability of the end organ to maintain normal function in the face of this intense stimulation, a deficiency related to the absence of carbohydrate active adrenal hormones.

SUMMARY

1. All symptoms of the circulatory failure which normally follow bilateral extirpation of the adrenal glands of the dog at a single operation, can be prevented by a thorough infiltration of the sympathetic elements adjacent to the glands prior to the adrenal removal. Anatomical dissection shows that the elements involved are the major parts of the coeliac plexus.

2. Spinal anesthesia and section of the spinal cord at T-1 or 2 affords comparable protection against the circulatory failure.

3. The circulatory failure following a twenty-five minute gentle stripping of the intestine can likewise be prevented by spinal section, spinal anesthesia, or thorough infiltration of the coeliac area with procaine.

4. It is concluded that trauma to visceral afferent elements in the splanchnic area, leading presumably to intense reflex stimulation of the peripheral vasculature via the vasomotor center, is an initiating factor in the production of circulatory failure in both intestinal stripping and bilateral adrenalectomy at a single stage operation.

REFERENCES

(1) SWINGLE, W. W., J. W. REMINGTON, V. A. DRILL AND W. KLEINBERG. This Journal (in press).
(2) REMINGTON, J. W., V. A. DRILL, W. KLEINBERG AND W. W. SWINGLE. Endocrinology (in press).
(3) FREUD, J., I. UYLDERT AND M. L. WATERMAN. Endocrinology 22: 497, 1938.
(4) STIEMENS, M. J. Verhand. Kon. Akad. Wetensch. Amsterdam 2: 33, 1934.
(5) PARKINS, W. M., W. W. SWINGLE, J. W. REMINGTON AND V. A. DRILL. This Journal 134: 426, 1941.
(6) THORN, G. W. AND H. EISENBERG. Endocrinology 25: 39, 1939.
(7) DE TAKATS, G. AND F. P. CUTHBERT. This Journal 102: 614, 1932.

THE RESPONSE OF SPLANCHNIC BLOOD VESSELS AND OF THE SMALL INTESTINE TO VASOCONSTRICTOR INFLUENCES IN ADRENAL INSUFFICIENCY IN THE CAT

J. L. A. FOWLER[1] AND R. A. CLEGHORN

From the Department of Medicine, University of Toronto, Toronto, Canada

The pressor action of pituitrin, barium chloride, nicotine and splanchnic nerve stimulation is greatly reduced in severe adrenal insufficiency in the cat, while that of epinephrine is but little impaired (1, 2, 3, 4, 5, 6). The congested state of the abdominal viscera, found at autopsy, in such animals suggested that a loss of contractility might have occurred in splanchnic blood vessels, which would account, at least in part, for the reduced pressor responses to pitressin and barium chloride (5). Since it had been found that epinephrine caused constriction of at least some of these vessels in adrenal insufficiency, the apparent ineffectiveness of splanchnic nerve stimulation and of nicotine, as judged by the effect on blood pressure, seemed anomalous. To explain this it was suggested that the store of sympathin at adrenergic nerve endings had become depleted (5). More recent work indicates that there is no substantial depletion of splanchnic sympathin in adrenalectomized cats up to the time of final collapse which was thought to be a result of cardiac failure (6).

In the present paper, further observations regarding the responsiveness of splanchnic vessels and, incidentally, of the smooth muscle of the small intestine of cats in adrenal insufficiency will be presented. A brief report (7) on this investigation was made in 1939.

METHODS. All observations were made on cats anesthetized with nembutal. Practically the whole length of the small intestine was placed in a plethysmograph containing cotton soaked in warm saline on which the bowel rested. The chamber of the plethysmograph was covered by a glass plate sealed by vaseline and connected to a Marey tambour which recorded intestinal volume changes. No stress is placed on minor differences found in gut volume changes in these experiments. Carotid blood pressure was also recorded, using a mercury manometer. (Parke, Davis and Company pitressin and Connaught Laboratories epinephrine were used.)

Experiments were carried out on three groups of animals: 1, on normal cats acutely adrenalectomized, i.e., immediately before the plethysmograph experiment; 2, on healthy adrenalectomized cats maintained by cortin for four to eighteen days; 3, on moribund, adrenalectomized cats dying of adrenal insufficiency.

RESULTS. 1. *Pressor responses.* The pressor responses elicited by stimulation of the right splanchnic nerve (in the abdomen) and by various pressor drugs in the three groups of adrenalectomized cats are listed in table 1. In adrenal

insufficiency the pressor responses, with the exception of that to epinephrine, were very much less than in the cats of groups 1 and 2.

The degree of impairment of the responses in the cats suffering from adrenal insufficiency was not so great as in many previously observed cases in which the gut was not plethysmographed (5). This was due to the slightly less advanced stage of adrenal insufficiency at which the present experiments were done. Waiting an hour or two till the condition became more severe would have greatly increased the risk of the animals dying before the more complicated plethysmograph set-up could be completed.

The significance of a generally smaller pressor response in cortin-treated cats than in the acutely adrenalectomized group is questionable in view of the limited number of experiments. Moreover, the smaller amount of nembutal required

TABLE 1

Pressor responses in adrenalectomized cats

CATS		Stimulation of rt. splanchnic nerve	AVERAGE INCREASE IN BLOOD PRESSURE FOLLOWING		
			Injection of drugs*		
Group	No.		Epinephrine, 0.04 mgm.	Pitressin, 2 units	Barium chloride, 25 mgm.
		mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*
Group 1:					
Acutely adrenalectomized..........	13	56	79	47	73
Group 2:					
Cortin-treated†	4	33	59	41	73
Group 3:					
Adrenal insufficiency..............	5	7	69	9	18

* Drugs injected intravenously by femoral vein over a ten-second period in volume of 1 cc.

† Maintained four to eighteen days.

to produce satisfactory anesthesia in the cortin-treated group may mean that depressor reflexes were not so effectively dampened. It is of interest that splanchnic nerve stimulation in cats under urethane anesthesia had a greater pressor effect in cortin-treated than in acutely adrenalectomized animals (6).

2. *Volume and vessel changes in the plethysmographed intestine.* The plethysmographed intestine showed the following changes elicited by the various procedures:

Splanchnic nerve stimulation: (1) *In the acutely adrenalectomized group:* A marked decrease in gut volume and visible constriction of vessels in the mesentery and intestinal wall occurred simultaneously with the rise in blood pressure (fig. 1a). Following the initial decrease in gut volume there was an increase above the resting level. In those cases in which the stimulation was prolonged for a minute or more, the increase in volume occurred before the stimulation was stopped. Increase in volume was associated with definite relaxation of the

bowel, though the visible vessels remained constricted. The volume of blood in non-constricted vessels probably was increased.

(2) *In the cortin-treated group:* The findings were similar to those in group 1, except that no secondary increase in gut volume and little or no relaxation beyond the resting level occurred.
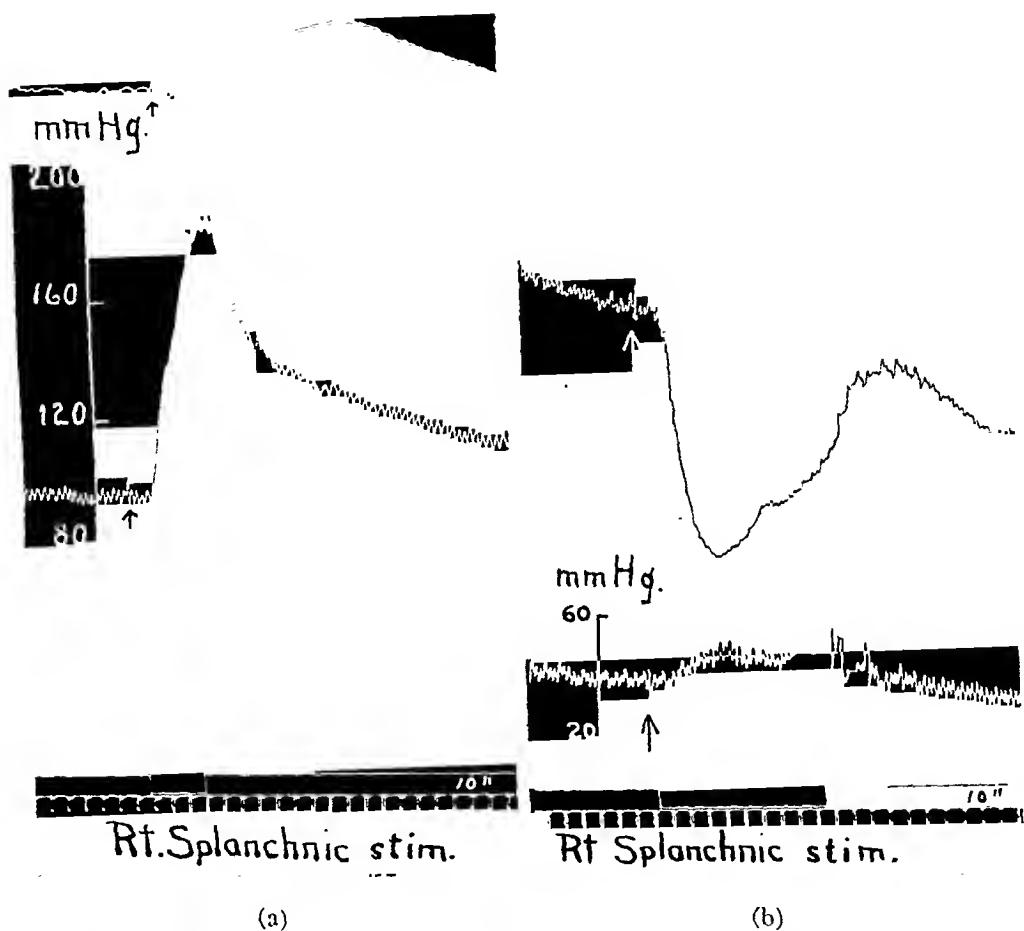
Fig. 1. Changes in volume of plethysmographed intestine (upper tracing) and pressor response (lower tracing) in adrenalectomized cats, in this and all other figures. Arrows indicate simultaneous points in time.) (a) Acutely adrenalectomized cat: right splanchnic nerve stimulated supramaximally in abdomen. (b) Cat in severe adrenal insufficiency: right splanchnic nerve stimulated supramaximally in abdomen.

(3) *In the adrenal insufficiency group:* The visible changes in vessels and the recorded decrease in gut volume appeared to be as marked as in the previous groups. There was no secondary increase in gut volume or relaxation of the intestine and little rise in blood pressure (fig. 1b).

Epinephrine: (1) *Acutely adrenalectomized group:* A marked decrease in gut volume and visible constriction of splanchnic vessels occurred three or four

seconds following the onset of a rise in blood pressure (fig. 2a). While the blood pressure was still elevated, the gut volume increased to a point considerably beyond the resting level. This was associated with obvious relaxation of the bowel.

(2) *Cortin-treated group.* All reactions were similar to those in the previous group.
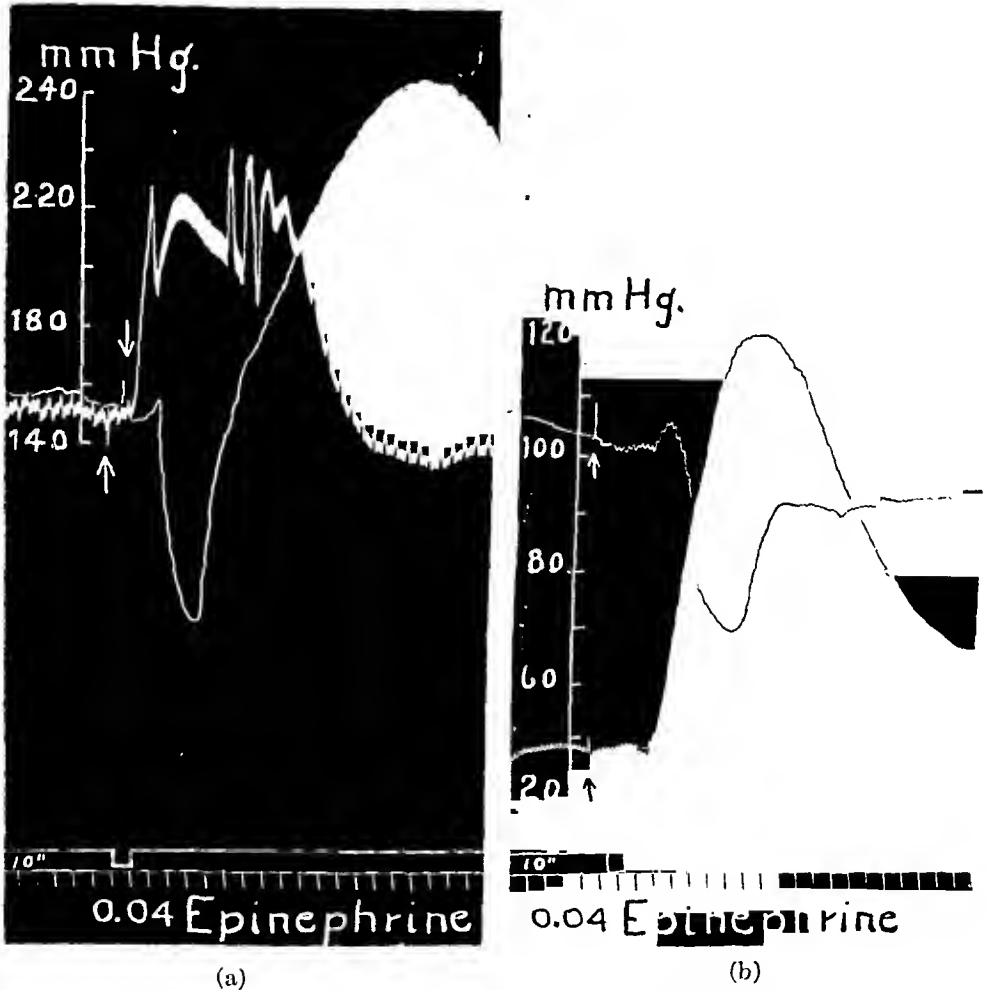
Fig. 2. (a) Acutely adrenalectomized cat: 0.04 mgm. epinephrine injected intravenously. (Same cat as fig. 1a.) (b) Cat in severe adrenal insufficiency: 0.04 mgm. epinephrine injected intravenously. (Same cat as fig. 1b.)

(3) *Adrenal insufficiency group.* The gut volume increased slightly simultaneously with, and probably as a result of, the initial rise in blood pressure. The blood pressure had risen considerably before constriction of the splanchnic vessels and decrease in gut volume occurred (fig. 2b). These changes were of the same type as those seen in the healthy groups, except that the time elapsing between the onset of the pressor effect and the splanchnic changes was more than twice as great in the moribund animals, indicating reduced circulation

time. The secondary increase in gut volume beyond the resting level seen in groups 1 and 2 did not occur in the adrenal insufficiency group despite the fact that the pressor response was marked. Only once was slight relaxation of the bowel observed.

Pitressin: The character and degree of changes in the vessels and intestine seemed to be the same in all three groups of cats. The diameter of the larger splanchnic arteries in the mesentery seemed to constrict first. Then, blanching and strong contraction of the intestinal wall developed and the gut volume fell.
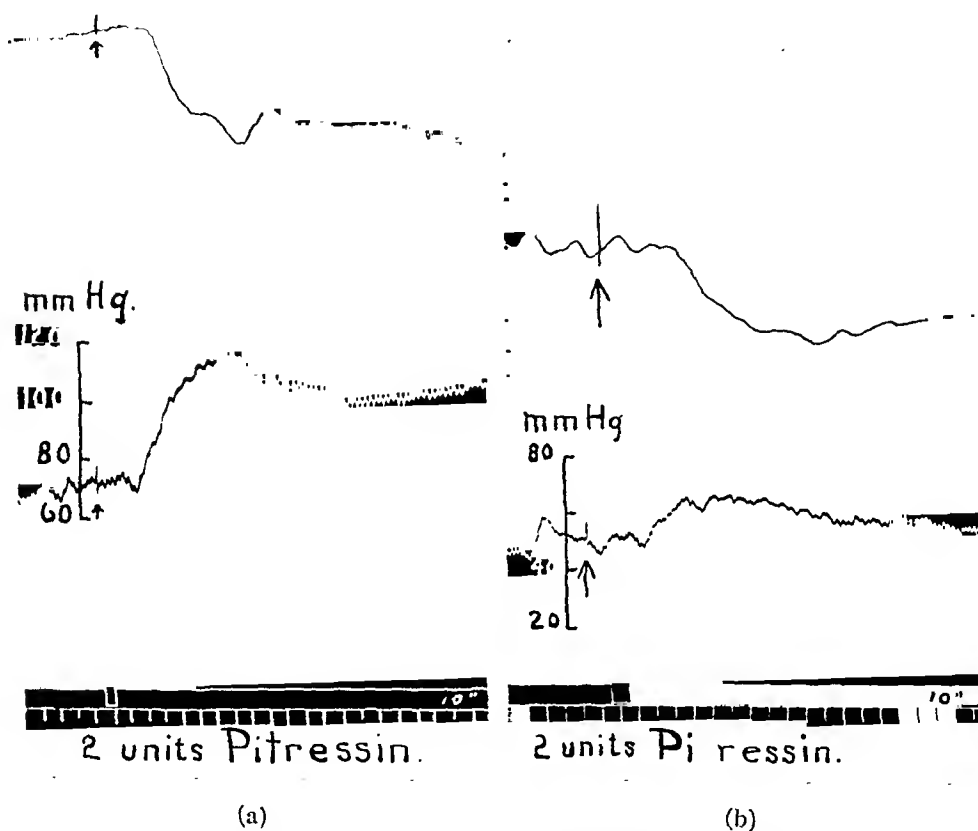
Fig. 3. (a) Acutely adrenalectomized cat: 2 units pitressin injected intravenously. (b) Cat in severe adrenal insufficiency: 2 units pitressin injected intravenously.

A slight increase in peristalsis was observed. The reduction in the gut volume was prolonged. The blood pressure had risen a little by the time the splanchnic vessels constricted. In the adrenal insufficiency group, the pressor effect was slower in developing and also less marked than in the other two groups. Figure 3 (a) illustrates the pressor effect and gut volume changes in an acutely adrenalectomized cat, and (b) in a cat in adrenal insufficiency.

Barium chloride: The changes in the plethysmographed intestine were the same in all three groups. The gut first became pale, and the volume decreased a few seconds after the onset of a rise in blood pressure. The volume decrease

was not so marked as with the other procedures, particularly in the cats that had previously received pitressin. The initial decrease in gut volume was followed in a few seconds by a profound and prolonged increase above the resting level. Similar observations on rabbits were made by White (8). The increase in gut volume was due to intense engorgement of intestinal vessels. The gut rapidly assumed a cyanotic hue. Concurrently with the vascular changes, local and variable contractions of the intestine were seen. Peristalsis increased, and as the gut became engorged and cyanotic it appeared to relax. Figure 4 (a and b) illustrates the pressor and graphically recorded plethysmograph changes.
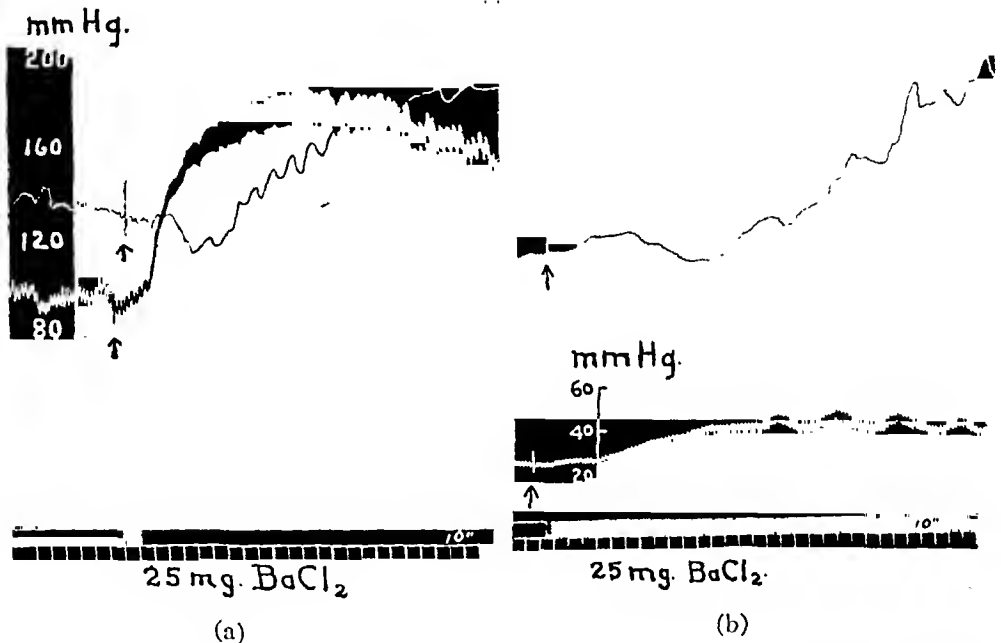
Fig. 4. (a) Acutely adrenalectomized cat: 25 mgm. barium chloride injected intravenously. (b) Cat in severe adrenal insufficiency: 25 mgm. barium chloride injected intravenously. (Both cats had received pitressin previously.)

It was also found that splanchnic nerve stimulation or the administration of epinephrine during the period of increased gut volume elicited by barium chloride caused a marked decrease in the volume.

3. *Other changes in the small intestine.* A noteworthy feature, which to our knowledge has not been remarked upon, is the highly contracted state of the small intestine of most cats dying of adrenal insufficiency. Since these animals eat little in the last few days of life, the gut generally is empty. In order to determine whether this was the reason for the contracted state and to insure that the controls were comparable in point of intestinal content, some of the animals in group 1 were starved for a number of days similar to the period of anorexia shown by cats developing adrenal insufficiency. These starved control animals all showed marked relaxation of the intestines. This indicates that the contracted intestine of animals dying in adrenal insufficiency is related to some other state associated with adrenal insufficiency. To determine whether

it was related to decreased blood volume, characteristic of adrenal insufficiency, a moribund cat with its gut in the plethysmograph was given 85 cc. physiological saline intravenously. Following this, the gut relaxed somewhat and the pulse pressure increased but the blood pressure only rose from 24 to 30 mm. Hg. Splanchnic nerve stimulation and the injection of epinephrine within eight minutes of the administration of saline each caused the usual reduction in gut volume, but this was followed by a marked increase in volume and by a relaxation of the intestine of the type seen in healthy adrenalectomized cats and not obtained in the animal dying of adrenal insufficiency, prior to the saline infusion. Relaxation of the intestine following splanchnic nerve stimulation can not have been due to the associated increase in blood pressure (10 mm. Hg) for this was no greater than that obtained by splanchnic nerve stimulation prior to the infusion of saline when no relaxation of the gut occurred. Circumstances prevented the repetition of this experiment.

Discussion. In the present study it has been shown that splanchnic blood vessels of cats in severe adrenal insufficiency constricted when the nerves supplying them were stimulated. Therefore, there was no exhaustion of sympathin at the adrenergic nerve endings in this region. The poor pressor response attending splanchnic nerve stimulation could not have been dependent on functional defect in the nerves or the vessels they supplied. This confirms findings previously reported (6) and also weighs against Secker's view (9, 10) that adrenalectomy leads to a loss of function of sympathetic vasoconstrictors.

Pitressin and barium chloride injected intravenously also caused constriction of vessels in the splanchnic region in adrenal insufficiency. The effect of barium chloride was predominantly on the veins. These observations rule out the hypothesis previously advanced (5) that loss of responsiveness of blood vessels to pitressin and barium chloride is the reason for the poor pressor effect following their injection in severe adrenal insufficiency. They also refute the allegation of inadequate dosage advanced by Remington et al. (11) as the explanation of this phenomenon.

The normal pressor effect elicited by epinephrine in adrenal insufficiency would seem to be due to the cardiac stimulating effect of this drug, since it did not produce greater constriction of mesenteric and intestinal wall vessels than splanchnic nerve stimulation or pitressin which caused so little rise in pressure. This view is supported by the observation of Clark (12) that the constriction, elicited by epinephrine, of the vessels of the intestine in normal cats is very transient. As a result of his studies, Clark concluded that increased cardiac output probably is the most important factor in the rise in blood pressure resulting from epinephrine action.

Splanchnic nerve stimulation, pitressin, and barium chloride cause no substantial pressor effect in adrenal insufficiency though, apparently, they exert the same marked degree of peripheral vasoconstriction as normally. This failure to cause the usual rise in blood pressure may be due to the inadequate response of the heart to increased peripheral resistance. Normally the heart responds to an increase in peripheral resistance by dilatation and an increase

in work done, while the blood pressure rises (13). In adrenal insufficiency, it would seem that the heart has lost the capacity to perform more work under the stimulus of increased resistance. Dilatation does not provoke discharge of the ventricular content. Visual inspection of the heart of animals dying in adrenal insufficiency shows it to be overly dilated and contracting feebly. It stops in diastole. The heart is implicated too in the cardiovascular failure by the fact that the terminal decline in blood pressure is associated with a decreasing heart rate (3).

This interpretation of the failure of increased peripheral resistance to cause a rise in blood pressure comparable to that seen in healthy adrenalectomized animals makes possible a re-interpretation of the findings of Remington et al. (11). These investigators say that their failure to obtain the usual pressor effect with renin and other pressor agents in adrenal insufficiency in dogs is due to disturbance of the "vascular peripheral apparatus," but they have not shown that there is any impairment in the power of these agents to constrict blood vessels. On the basis of our findings, it seems probable that their results may have been due to a failure of the heart to respond to the stimulus of increased peripheral resistance in the same manner as in healthy controls.

It should be noted that the pressor drugs employed all caused the blood pressure to rise before causing constriction of splanchnic blood vessels. This is attributable to an effect on those parts of the cardiovascular tree first reached by these drugs, e.g.: the pulmonary system and, in the case of epinephrine, the heart as well and more particularly.

The increase in volume of the plethysmographed intestine, following the preliminary decrease in response to epinephrine, observed in our healthy animals has been described by White (8) and Clark (12) in non-adrenalectomized cats. In Clark's opinion it is due to an increase in the amount of blood in the intestine where constriction does not last as long as, for example, in the skin. It is a result largely of passive dilatation of the vessels in that region due to the raised blood pressure. Section of the splanchnics excluded depressor reflexes as a cause. The observations of White indicate that a persistence of constriction of mesenteric veins after relaxation of arterioles is an important factor in the increase in intestinal volume with epinephrine. In some of his experiments— as in ours, in adrenal insufficiency—no secondary increase in volume occurred. In such cases White observed that arterioles and veins recovered simultaneously from the constricted state.

Our experiment on a cat in adrenal insufficiency, in which the intravenous injection of a large amount of physiological saline restored the intestinal responses to normal, indicates that either the reduced blood volume or a disturbance in the ionic ration of sodium, chloride and potassium in the gut was responsible for the contracted state of the intestine and abnormal responses.

SUMMARY

In cats dying of adrenal insufficiency, splanchnic nerve stimulation or the intravenous injection of pitressin or barium chloride constricts blood vessels

in the splanchnic region as in healthy adrenalectomized controls. The pressor response to these procedures in adrenal insufficiency was poor while that elicited by epinephrine was practically unimpaired. The retention of the pressor effect by epinephrine is attributed to its cardiac stimulating action. It is concluded that splanchnic nerve stimulation, pitressin and barium chloride cause but poor pressor response in adrenal insufficiency because the heart fails to respond normally to an increase in peripheral resistance.

The contracted state of the small intestine in moribund adrenalectomized cats is remarked upon and the absence of relaxation attending splanchnic nerve stimulation or the injection of epinephrine. Evidence indicating that this abnormal reaction may be related to blood volume deficiency or to alteration in blood electrolytes is presented.

REFERENCES

(1) ELLIOTT, T. R. J. Physiol. 31: 20P, 1904.
(2) ELLIOTT, T. R. J. Physiol. 49: 38, 1914.
(3) CLEGHORN, R. A. Proc. XVI Internat. Physiol. Cong., Kongressbericht 2: 206, 1938.
(4) CLEGHORN, R. A. This Journal 128: 133, 1939.
(5) ARMSTRONG, C. W. J., R. A. CLEGHORN, J. L. A. FOWLER, AND G. A. McVICAR. J. Physiol. 96: 146, 1939.
(6) CLEGHORN, R. A., C. W. J. ARMSTRONG, D. C. AUSTEN, AND G. A. McVICAR. This Journal 132: 542, 1941.
(7) CLEGHORN, R. A., J. L. A. FOWLER, AND J. S. WENZEL. This Journal 126: 466, 1939.
(8) WHITE, A. C. J. Pharmacol. and Exper. Therap. 32: 135, 1927.
(9) SECKER, J. J. Physiol. 89: 296, 1937.
(10) SECKER, J. J. Physiol. 94: 259, 1938.
(11) REMINGTON, J. W., W. D. COLLINGS, H. W. HAYS, W. M. PARKINS, AND W. W. SWINGLE: This Journal 132: 622, 1941.
(12) CLARK, G. A. J. Physiol. 69: 171, 1930.
(13) KATZ, L. N., K. JOCHIM, E. LINDNER, AND M. LANDOWNE. This Journal 134: 636, 1941.

A STUDY ON THE BLOOD VOLUME OF A GROUP OF UNTRAINED NORMAL DOGS

D. D. BONNYCASTLE and R. A. CLEGHORN

From the Departments of Pharmacology and Medicine, University of Toronto, Canada

This report deals with the results of blood volume estimations carried out during the course of experiments investigating some aspects of shock. The animals used were unselected and untrained dogs of mixed sex and breed. The literature concerning the estimation of blood volume in dogs has been dealt with adequately in the papers referred to below.

METHODS. Plasma volume was measured by the use of the dye T 1824 in a sterile 1 per cent solution (1). A standard concentration curve was prepared as described previously (2), using the Evelyn photoelectric colorimeter.

The animals used had been in the laboratory on an average of a week. They varied in weight from 4 to 19 kgm.; only 5 were more than 13 kgm. The dogs were fasted 15 to 18 hours before the blood volume estimation was carried out. No sedative or anesthetic was used. At the beginning of each experiment a control sample of blood (3–4 cc.) was drawn under oil from a leg vein, and the dye was then injected, the amount used in most cases being 1 cc. of the 1 per cent dye solution diluted with saline to 8–10 cc. Further blood samples were taken at 30, 60 and 90 minute intervals from one of the opposite leg veins. This interval of 30 minutes before taking the first dye sample allowed thorough mixing of the dye to take place, and its disappearance rate to become uniform.

The density values of the dye T 1824 in the various samples were determined on 0.5 cc. serum samples, using filter 635 in the Evelyn photoelectric colorimeter and a correction for any possible hemolysis was made according to the method described by Gibson and Evelyn (3). Estimations of the packed volume of red blood cells were made on heparinized blood. The animals were not tied to a table during the determination but left in a cage. Care was taken to avoid excitement by careful handling. Some animals were nevertheless excited at first and in these the initial packed cell volume was considerably higher than in subsequent samples, presumably due to this cause. In such cases the initial value was discarded and an average of the lower subsequent values taken as the per cent cell volume for this paper.

The red cell volume was calculated by use of the formula

$$\frac{\text{Plasma volume} \times \text{Per cent cell volume}}{\text{Per cent plasma volume}} = \text{Red cell volume.}$$

Total blood volume was obtained by adding the red cell and plasma volumes together. The experiments were done during the months November to April.

RESULTS. The plasma volume and red cell volume in terms of cubic centimeters per kilogram of body weight, together with the per cent cell volume

TABLE 1

Plasma and cell volume in 106 normal dogs

DOG NO.	SEX	WEIGHT (kgm.)	PACKED VOLUME R.B.C.	BLOOD PLASMA (cc./kgm.)	VOLUME RED CELL (cc./kgm.)	DOG NO.	SEX	WEIGHT (kgm.)	PACKED VOLUME R.B.C.	BLOOD PLASMA (cc./kgm.)	VOLUME RED CELL (cc./kgm.)
1	F	6.06	44	42.7	33.7	54	F	6.65	44	47.8	37.6
2	M	9.4	48	43.6	40.3	55	F	6.88	39	44.2	27.8
3	F	10.5	42	48.9	35.5	56	F	5.8	50	38.4	38.4
4	F	8.75	39	60.7	38.5	57	F	7.0	42	46.2	33.5
5	F	10.4	29	54.6	24.3	58	F	4.7	41	48.0	33.4
6	M	8.8	38	60.1	36.5	59	F	6.95	43	38.8	28.1
7	M	8.45	44	49.4	38.8	60	F	6.67	41	49.8	34.5
8	F	10	44	50.8	39.9	61	M	9.9	47	42.2	37.4
9	F	6	37	60.1	36.2	62	F	8.15	35	48.0	25.8
10	F	7.9	45	42.1	35.2	63	F	9.2	46	57.9	49.2
11	M	9.34	41	51.0	32.1	64	M	7.78	43	45.5	34.4
12	M	10.09	45	51.0	40.9	65	M	8.3	46	46.6	39.8
13	F	6.3	40	64.6	43.0	66	F	12.25	44	35.0	27.4
14	F	6.9	45	57.9	47.4	67	M	7.93	46	36.8	31.4
15	F	6.6	45	55.1	45.2	68	M	15.5	36	42.3	23.8
16	M	9.35	42	54.9	40.5	69	F	7.23	47	48.2	42.6
17	F	6.5	45	38.0	30.9	70	M	7.28	46	55.0	46.6
18	F	8.5	45	45.4	37.4	71	F	8.3	40	49.2	32.8
19	M	11.9	40	52.1	35.5	72	F	5.5	50	44.3	44.3
20	?	11.7	40	51.5	40.5	73	F	7.0	31	54.5	24.3
21	F	7.47	45	54.5	44.6	74	M	10.6	40	48.0	31.1
22	M	11.75	46	54.7	46.4	75	M	6.38	38	47.8	29.7
23	M	13.2	37	52.9	31.7	76	M	6.16	43	52.4	39.6
24	M	14.4	40	56.0	38.0	77	F	11.7	47	31.8	28.2
25	F	9.4	41	34.4	23.9	78	M	12.55	44	37.1	29.7
26	F	10.2	41	46.5	28.5	79	F	12.25	33	62.7	30.8
27	F	10.8	46	43.2	37.6	80	M	11.57	33	62.8	30.8
28	F	8.9	35	40.1	22.0	81	M	7.95	49	38.1	36.7
29	F	7.8	41	50.2	35.6	82	F	8.62	45	42.4	34.7
30	M	8.6	41	53.7	38.1	83	F	10.9	49	32.4	31.1
31	F	7.6	39	55.0	35.1	84	M	10.3	48	32.9	30.4
32	F	7.6	52	42.9	46.5	85	F	19.5	35	39.0	21.0
33	M	9.2	44	40.5	31.7	86	F	6.19	49	40.5	39.0
34	F	8.9	47	43.6	38.6	87	M	7.5	41	46.7	32.5
35	F	9.5	39	42.7	27.3	88	F	6.75	45	38.4	31.4
36	F	9.3	44	48.2	37.9	89	M	7.87	45	50.8	41.7
37	F	6.8	45	48.3	39.4	90	F	6.67	44	54.0	42.5
38	M	9.8	47	54.6	48.5	91	M	8.29	39	56.4	36.0
39	F	17.2	46	40.5	34.5	92	F	7.0	44	55.6	43.7
40	F	6.9	39	54.4	34.8	93	F	7.59	44	39.4	31.0
41	F	9.1	43	44.5	33.6	94	F	8.87	29	57.0	23.3
42	F	8.7	37	49.4	29.6	95	M	7.83	42	51.3	37.3
43	F	8.35	40	44.9	29.9	96	M	9.46	41	48.1	33.4
44	F	6.25	42	56.8	41.1	97	M	10.09	41	47.3	32.7
45	M	7.4	38	51.8	31.8	98	M	7.5	39	42.5	27.2
46	F	6.3	43	48.9	36.9	99	F	6.6	50	37.0	37.0
47	M	7.8	39.5	45.4	29.6	100	M	6.07	39	57.0	36.0
48	F	7.6	40	48.7	33.2	101	F	11.56	28	54.1	21.1
49	F	9.93	38	61.3	36.8	102	F	8.3	38	50.9	31.2
50	M	11.03	36	57.3	32.2	103	F	5.5	44	46.7	36.6
51	F	7.35	39	48.0	30.7	104	M	7.17	45	43.9	35.9
52	M	9.8	40	48.4	32.3	105	M	9.4	48	40.5	38.2
53	F	4.7	42	47.0	34.0	106	M	12.1	45	49.8	41.4

values from estimations on 106 different animals weighing from 4.7 to 19.5 kgm., are given in table 1.   Figures 1 and 2 present the frequency distribution of the plasma volumes and total blood volumes expressed as cubic centimeters per kilogram.

The range of plasma volumes found was 31.8 to 64.6 cc./kgm., of cell volume 21 to 49.2 cc./kgm. and for total blood volume 60 to 107.5 cc./kgm.   Our findings with regard to plasma volume correspond very closely to those reported by Gregersen and Stewart (1), who with the same dye method obtained a range of 35 to 65 cc./kgm. in a large series of animals; no calculations of total blood volume were made in their report.

Gibson, Keeley and Pijoan (4) have described results of plasma volume studies in 50 dogs weighing from 5 to 30 kgm.   They also used the dye T 1824 and obtained values for plasma volume ranging from 41.2 to 51.7 cc./kgm., for
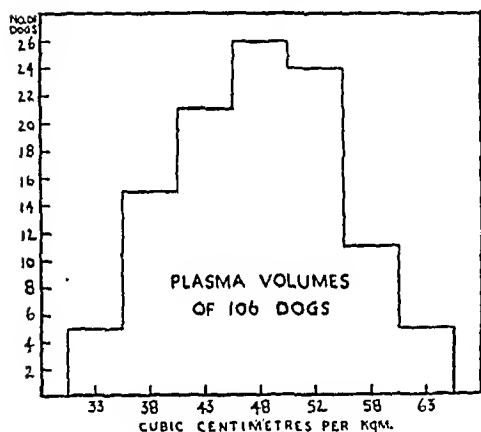
Fig. 1
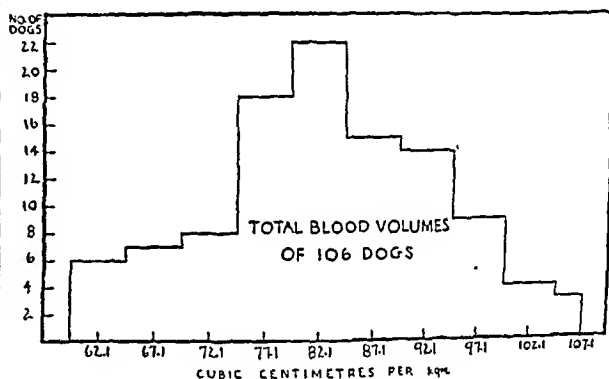
Fig. 2

Fig. 1. Frequency distribution of plasma volumes of 106 unselected dogs. (Median 48 cc./kgm.)

Fig. 2. Frequency distribution of total blood volumes of 106 unselected dogs. (Median 82.1 cc./kgm.)

cell volume 36.4 to 54.6 cc./kgm. and for total blood volume 84 to 97.3 cc./kgm. They also showed that the larger dogs tended to have a larger plasma, cell and total blood volume per kgm. body weight.   The great majority of our animals weighed less than 13 kgm.   When our results are compared to those on the 14 animals of Gibson et al. which weighed less than 13 kgm., the plasma volumes agree very closely, the averages within 1 per cent.   Our cell volumes were on the whole lower than those obtained by these workers, our average value being 34.1 cc./kgm., theirs 40.5 cc./kgm. on the 14 dogs under 13 kgm.   If they used jugular (source of venous blood not stated) rather than leg vein blood, their higher result would be explained, in part at least, since the packed red cell volume is greater in the larger than in the smaller vessels (1).

No consistent differences were observed between the average cc./kgm. value of plasma and total blood volumes between male and female animals in our experiments, in agreement with the observations of the above authors (4).

The spread of values in plasma volume and total blood volume is shown in the graphs, and the figures assume the configuration that might be expected in a group of animals, unselected with respect to sex, age, breed, muscularity or adiposity, factors which are suspected as affecting the unit volume of plasma and total blood.

SUMMARY

Plasma volume estimations were carried out on 106 normal, unselected dogs of from 4.7 to 19.5 kgm., only 5 of which were more than 13 kgm. In terms of cubic centimeters per kilogram, plasma volume ranged from 31.8 to 64.6 cc., cell volume from 21 to 49.2 cc. and total blood volume from 60 to 107.5 cc. No significant difference was noted between the average cc./kgm. value of plasma and total blood volumes between males and females.

REFERENCES

(1) GREGERSEN, M. L., AND J. G. STEWART. This Journal **125**: 142, 1939.
(2) BONNYCASTLE, D. D. J. Pharmacol. and Exper. Therap. (in press).
(3) GIBSON, J. G. AND K. A. EVELYN. J. Clin. Investigation **17**: 153, 1938.
(4) GIBSON, J. G., J. L. KEELEY AND M. PIJOAN. This Journal **121**: 800, 1938.

THE DISTRIBUTION OF WATER AND ELECTROLYTES BETWEEN BLOOD, FLUIDS AND SKELETAL MUSCLE IN PREGNANT DOGS[1]

ALICE CHILDS AND LILLIAN EICHELBERGER

From the Department of Medicine and Lasker Foundation for Medical Research of the University of Chicago

Since considerable work has been done on the water equilibria in pregnancy, it was supposed that a study of the distribution of water and electrolytes in muscle from pregnant dogs would provide useful data. The distribution of water and electrolytes in normal mammalian muscle having been established, a study of the muscle removed from pregnant dogs would define the relative proportions of extracellular and intracellular water in the pregnant organism. This report, therefore, will present data to show first, that the concentration of electrolytes and the volume of extra and intracellular fluids of skeletal muscle from pregnant dogs were not different from those in the non-pregnant organism; and second, that following an increase in total body water, the average acute tissue edema produced in the pregnant organism was of the same proportion as that produced in the normal non-pregnant animal.

METHODS. Dogs were used for this study. They were either obtained in late pregnancy and kept in the laboratory for a few days on a meat diet, or they were obtained in early pregnancy and kept for a number of weeks. Before an experiment was carried out, the dog was weighed and anesthetized by the intravenous injection of 1 cc. per kilo body weight of a 5 per cent solution of nembutal. A cannula was then introduced in the carotid artery for continuous blood pressure tracing and the bladder was catheterized for the collection of urine during the entire experimental period. Forty cubic centimeters of blood were then removed by syringe from the femoral artery, of which 30 cc. were delivered under oil for serum analysis, and 10 cc. were defibrinated for whole blood analysis. One rectus abdominis muscle was removed for the initial analyses and placed immediately in a large glass-stoppered weighing bottle. It was then placed on a glazed tile and trimmed to remove all visible connective tissue and fat, after which it was returned to the weighing bottle. After being minced with scissors the muscle was ready to be analyzed. Weighed aliquot samples were used for all analyses.

The salt solution consisting of 129 mM NaCl + 25 mM NaHCO$_3$ was warmed to 38° and injected intravenously by gravity through the femoral vein at a speed of 40 cc. per minute. The injection required about one hour, and 30 minutes later the second sample of blood was withdrawn, and the opposite rectus abdominis muscle removed for final analyses.

---

[1] This communication is a portion of a thesis submitted by Alice Childs in partial fulfillment for the degree of Doctor of Philosophy in Medicine at the University of Chicago. A preliminary report has appeared. (Proc. Am. Soc. Biol. Chem. 1942.)

The peritoneal fluid, amniotic fluid and allantoic fluid were then removed. Special care had to be taken in removing first the fluid from the allantoic sac and then the fluid from the amniotic sac surrounding each fetus. The procedure was as follows: An incision was made along the mid line of the dog and the horns of the uterus were exposed. After an incision was made through the wall of the uterus the allantoic sac surrounding a fetus was visible. With a needle and syringe the fluid from the sac was removed, after which the allantoic membrane was stripped away and the membrane surrounding the amniotic sac was exposed. This membrane was wiped with gauze and the amniotic fluid removed with needle and syringe. Each fetus was treated in this manner. In late pregnancy, the volume of allantoic fluid around each fetus was generally larger than that of the amniotic fluid. Each dog carried from 5 to 7 fetuses. For analysis, all of the fluid was removed from each of the individual sacs and mixed together.

There is a well developed allantois in the dog which is present through the entire gestation period so that both amniotic and allantoic fluids are available for study. According to the investigations of Bremer (1) the allantois is largest in those animals in which the fetal circulation is less intimately in contact with the maternal circulation and in which the mesonephric glomeruli are well developed and active. On this basis the dog is classified midway between the pig and man.

The study included: (1) 10 pregnant dogs from which blood, muscle, and fluids were analyzed; (2) 10 pregnant dogs receiving intravenous injections of 170 cc. per kilo of body weight of an isotonic salt solution consisting of 129 mM of NaCl + 25 mM of Na HCO$_3$.

Chemical methods. The following determinations were made on serum: pH, CO$_2$, water, protein, chloride, sodium, potassium, calcium and magnesium; on blood: hematocrit, water, chloride, sodium and potassium; and on amniotic and allantoic fluids: water, protein, chloride, sodium, potassium, calcium and magnesium. All analyses of serum and fluids were made in duplicate, all muscle analyses in triplicate. All tissue analyses were calculated on a fat-free, blood-free basis. The fat was determined as described in a former paper (2). The amount of the circulatory space of the blood in the muscle was determined by the colorimetric comparison of the amount of hemoglobin in the muscle with that of the whole blood taken as nearly simultaneously as possible, which was used as the standard (3). Procedure: Two grams of minced muscle were transferred to a 25 cc. volumetric glass-stoppered cylinder. Three-tenths cubic centimeter of a thirty-three per cent ammonium hydroxide was added and the mixture made up to volume with water and placed in a 2° to 5° ice box overnight. The mixture was then filtered through no. 40 Whatman filter paper into a flask. Three drops of concentrated hydrochloric acid were added to the clear filtrate while being shaken. The flask was stoppered and allowed to stand at room temperature for 1 hour. The acid hematin produced was read in a colorimeter against that produced from 0.5 cc. blood made to 100 cc. volume with 0.1 N HCl. The value for the circulating space so obtained is high because some muscle

hemoglobin as well as blood hemoglobin is extracted.   It is interesting to note that the value obtained for the vascular space in muscle from pregnant dogs ranged from 72 cc. to 114 cc. per kilo of muscle with a mean of 91 cc. per kilo while the mean circulatory space of muscle from non-pregnant dogs was 71 cc., $\sigma \pm 11$ per kilo.   The chemical methods for blood, fluids, and muscle were essentially the same as those described in previous papers (2, 4, 5).

Calculations.   The volumes of extra and intracellular phases of muscle were calculated as outlined in the first paper of this series (6).   The extracellular phase (F) in grams per kilo of muscle,

$$(F) = \frac{(Cl)_M \times (H_2O)_s \times 1000}{1.04 \times (Cl)_s}$$

in which subscripts M and s represent muscle and serum, respectively.   From the values for (F) the intracellular phase (C) per kilo was estimated by the equation $(C) = 1000 - (F)$.   From the values for (C) the intracellular water

TABLE 1

Water and electrolyte content of blood scrum and cells in late pregnancy

The concentrations are expressed per kilo of serum, and per liter of cells.

	SERUM		CELLS	
	Mean	σ	Mean	σ
pH.................................	7.29	0.05		
CO₂, mM...........................	21.99	2.1		
Water, gm.........................	927.0	3.9	714.4	12.3
Chloride, m.eq....................	108.5	1.9	60.0	5.8
Sodium, m.eq......................	137.9	2.8	97.0	9.0
Potassium, m.eq...................	4.30	0.50	11.7	2.3
Calcium, m.eq.....................	4.36	0.10		
Magnesium, m.eq..................	2.58	0.68		
Cell volume†			31.9	3.0
NPN, gm...........................	0.26	0.07		
Protein, gm.......................	54.1	4.3		

σ = Standard deviation.

† = cubic centimeter per 100 cc.

$(H_2O)_c$ was estimated by the equation $(H_2O)_c = (C) - (S)$ in which (S) represents solids per kilo of muscle.

RESULTS AND DISCUSSION.   *Analytical data on serum, blood, and muscle of pregnant dogs.   Values for constituents of serum and cells.*   A summary of the data obtained from the analysis of serum and the cells of 10 dogs in late pregnancy is presented in table 1.   The cell values were obtained by the indirect analyses of whole blood.   The differences between these data and those from normal dogs are a lower calcium in the serum, and a low cell volume.   All other values fall within the range of normal findings.   The value of 31.9 ± 3.0 for cell volume may be the result of the increased blood volume that generally occurs in pregnancy (7).   Although the blood volumes of these dogs were not deter-

mined, the determined average circulatory space in the rectus abdominis muscle was 30 per cent larger than in normal dogs, indicating an increased blood volume.

Values for constituents of muscle. In table 2 are the analytical and calculated data on serum and muscle from both non-pregnant and pregnant dogs. The relative proportions of extra and intracellular phases of muscle were calculated from the experimental data and by the foregoing equation. From these data the percentage of water per kilo of muscle cells $(H_2O)_c$ was also calculated. In view of the physical changes that occur in pregnant animals it is interesting to note that the concentration of the serum and muscle constituents of pregnant dogs was not different from that of normal dogs. Also, the distribution of fluids in the skeletal muscle was alike.

Conclusion: Fat-free, blood-free skeletal muscle of dogs in late pregnancy consists of an extracellular phase amounting to an average of 15 per cent and an intracellular phase amounting to 85 per cent of the muscle. These values

TABLE 2

Average analyses of skeletal muscle and serum from non-pregnant and pregnant dogs

DOGS		H_2O	Cl	Na	K	BLOOD	(F)	(S)	$(H_2O)_c$
		gm. per kgm.	*mM per kgm.*	*mM per kgm.*	*mM per kgm.*	*cc. per kgm.*	*gm. per kgm.*		*gm. per kgm. cells*
Non-pregnant (20)	Serum	922.0	107.1	141.2	3.95				
	σ	5.1	2.3	3.5	0.40				
	Muscle	775.0	18.41	29.0	98.4	71	154	225	736
	σ	8.6	3.6	6.0	7.5	11	19	8.6	9
Pregnant (10)	Serum	927.0	108.5	137.9	4.30				
	σ	3.9	1.9	2.8	0.50				
	Muscle	776.4	18.21	24.4	99.2	97	154	223	737
	σ	6.4	3.4	4.8	6.0	29	31	.6	8

σ = Standard deviation.

were the same as those found in non-pregnant dogs (fig. 1). In other words, there was no edema, either extra or intracellularly, in the skeletal muscle of the pregnant dogs.

Increase in total body water by the injections of normal isotonic salt solutions. In table 3 is presented the average water and electrolyte distribution in serum and muscle of pregnant dogs before (control) and following (experimental) the increase in total body water. Preceding the acute edema, both the blood serum and muscle picture were like that of non-pregnant dogs. Also, the analytical data of the experimental muscle following the injection of 170 cc. per kilo body weight of a solution containing 25 mM of $NaHCO_3$ + 129 mM of NaCl per liter, are given. From these experimental results the changes of the original muscle phases on the basis of a constant solid content of the intracellular phase were calculated. Such a calculation gave an average increase of 53 grams, σ ± 20 grams per kilo of original muscle of which 45 grams, σ ± 20 represent an increase in the extracellular phase and 8 grams, σ ± 10, an increase in the intracellular phase.

In non-pregnant animals receiving the same injection, the average increase in the total original kilo of muscle was 49 grams, $\sigma \pm 8$; the average increase of the extracellular phase was 53 grams, $\sigma \pm 18$. These findings are shown graphically in figure 1.

Therefore, in these experiments in which the total body water of the pregnant animal was increased by the intravenous injection of normal isotonic saline, there was no indication of an influence of pregnancy on the amount of acute edema formed in the skeletal muscle.

Although this pregnant organism contained two distinct gestation sacs filled with fluids, the skeletal muscle fluid distribution was the same as in normal dogs.

TABLE 3

Changes of water and electrolytes in serum and muscle of pregnant dogs after injection of normal isotonic sodium chloride solutions

Solution = 129 mM NaCl + 25 mM $NaHCO_3$. All concentrations are expressed in units per kilo.

		CONTROL		EXPERIMENTAL	
		Mean	σ	Mean	σ
Serum	pH	7.39	0.05	7.44	0.07
	CO_2, mM	21.99	2.1	22.31	1.4
	Water, gm.	927.0	3.9	951.2	6.7
	Cl, m.eq.	108.5	1.9	115.4	2.4
	Na, m.eq.	137.9	2.8	144.4	2.6
	K, m.eq.	4.30	0.50	3.38	0.53
Muscle	Water	776.4	6.4	788.2	9.5
	Cl, m.eq.	18.21	3.4	22.69	4.6
	Na, m.eq.	24.4	4.8	32.9	5.8
	K, m.eq.	99.2	6.0	89.9	1.0
	Blood, cc.	97.3	29.7	87.7	10.9
	(F), gm.	154.0	31.0	187	42
	{H_2O}c, gm.	737.0	8.0	739.0	6.7
	(S), gm.	223	6.0	211	7.6

σ = Standard deviation.

In table 4 are given the average analyses of all fluids as removed from the pregnant dog. The peritoneal and amniotic fluids are somewhat alike and their constituents have small deviations. The allantoic fluid is entirely different, with its constituents having large deviations. Since the allantoic fluid when compared with the other fluids had a very high concentration of potassium, it was expected that the concentration of potassium in the muscle cells of the pregnant dog might be lower than that found in the muscle of the non-pregnant dog. Table 5 shows this is not true. Both the analytical and derived data with standard deviations for 10 dogs for the distribution of potassium in serum, fluids and skeletal muscle of normal and pregnant dogs are given.

The concentration of potassium in (F) was estimated from the concentration

in serum water by means of the Hastings *et al.* and Greene *et al.* factor (0.95) (8, 9). From the amount of estimated phases per kilo of muscle and the calculated concentration of the potassium in the extracellular fluid, the concentration of potassium in the intracellular phase can be derived by subtracting the extra-
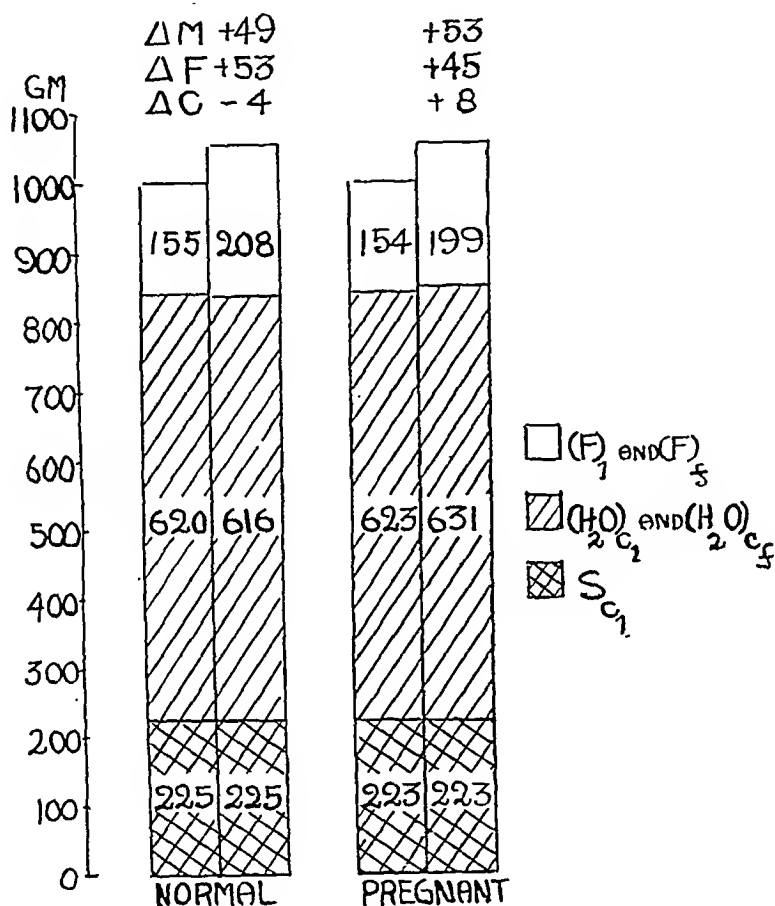
Fig. 1. Extracellular phase (F), intracellular water $(H_2O)_c$ and solids $(S)_{ci}$ before and after experimental procedure. The first column in each pair presents the average original data and the adjoining column the average final data from a group of normal and pregnant dogs. $\triangle M$ represents the absolute change in 1 kilo of muscle; $\triangle F$ and $\triangle C$, the absolute change in the extra and intracellular phase per kilo of original muscle respectively; $(F)_i$ and $(F)_f$, grams of extracellular phase per kilo of muscle, originally and finally, respectively; $(H_2O)_{ci}$ and $(H_2O)_{cf}$, grams of intracellular water per kilo of muscle, originally and finally, respectively; $(S)_{ci}$, grams of intracellular solids per kilo of muscle, originally.

cellular potassium from the total tissue potassium. The concentration of potassium per kilo of muscle cells was calculated by dividing the intracellular potassium by the intracellular phase volume, and the concentration of potassium in the intracellular water by dividing the intracellular potassium by the volume of intracellular water. The potassium concentrations in the skeletal muscle are given per kilo of intracellular phase (C) and also per kilo of cell water $(H_2O)_c$.

TABLE 4

The distribution of electrolytes between serum, peritoneal fluid, amniotic fluid and allantoic fluid

	SERUM		PERITONEAL FLUID		AMNIOTIC FLUID		ALLANTOIC FLUID	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ
Water, grams per kilo..........	927.0	3.8	985.0	1.7	984.9	1.9	977.6	10.1
Cl, m.eq. per kilo water........	117.0	1.9	124.4	2.2	113.2	2.5	60.7	20.6
Na, m.eq. per kilo water........	148.7	2.8	152.2	6.8	141.2	5.3	97.8	37.8
K, m.eq. per kilo water........	4.64	0.50	3.47	0.58	4.27	0.73	48.1	32.8
Ca, m.eq. per kilo water.......	4.71	0.10			6.02	0.48	3.78	0.68
Mg, m.eq. per kilo water.......	2.78	0.68			2.32	0.74	3.20	0.44
Non-protein nitrogen, grams per kilo water..................	0.28	0.07	0.19	0.03	0.37	0.11	1.47	1.66
Total protein, grams per kilo water......................	58.4	4.3	8.4	3.2	3.55	1.6	1.42	1.40

TABLE 5

Distribution of potassium between blood plasma and muscle phases

	NON-PREGNANT	PREGNANT
	m.eq.	*m.eq.*
Serum:		
Per kilo................................	4.09	4.30
σ................................	0.19	0.50
Per kilo H_2O...........................	4.41	4.66
σ................................	0.24	0.58
Per kilo interstitial fluid..................	4.19	4.42
σ................................	0.18	0.50
Muscle:		
Per kilo................................	97.1	100.9
σ................................	7.5	5.3
In (F)................................	0.54	0.60
σ................................	0.15	0.12
In (C)................................	96.6	100.3
σ................................	7.5	5.3
Per kilo (C)...........................	110.5	118.1
σ................................	7.0	4.4
Per kilo $(H_2O)_C$........................	151.0	160.4
σ................................	9.0	6.1

The results show that the potassium concentrations in the pregnant muscle are not different from those found in the non-pregnant muscle.

It is apparent, therefore, from a comparison of the data on non-pregnant dogs with those from the pregnant dogs that there is no edema of the skeletal

muscle in normal pregnancy, and if additional water is added to the pregnant organism it is distributed as in the normal non-pregnant animal. These findings substantiate the results of Chesley and Chesley (10) that in the normal pregnant woman, the extracellular water of the body was not excessive. Therefore, since edema does not accompany normal pregnancy, the findings in our dogs are in agreement with clinical experience.

It might have been reasonable to expect a shift in the distribution of the fluid in the skeletal muscle to provide for the extra volume of fluids filling the amniotic and allantoic sacs surrounding the fetuses. This was not verified. The negative findings again demonstrate the constancy with which the fluid of the muscle is distributed.

SUMMARY

1. Analyses have been made of the water and electrolytes of blood, muscle and the gestation fluids from a group of 10 dogs in the late weeks of pregnancy.

2. The investigation of the water and electrolyte distribution between blood and skeletal muscle of dogs in late pregnancy revealed the following: 1, the skeletal muscle consists of an extracellular phase amounting to an average of 15 per cent and an intracellular phase amounting to an average of 85 per cent of the muscle. These values were the same as those found in non-pregnant dogs; 2, after an increase in total body water produced by the intravenous injection of large volumes of normal isotonic salt solution, the amount of acute edema produced in the skeletal muscle was not different from that found in the non-pregnant organism.

3. There is, in these experiments, no indication of an influence of normal pregnancy upon the distribution of fluid in skeletal muscle and consequently no evidence for the occurrence of edema as a result of this state.

REFERENCES

(1) BREMER, J. L. Am. J. Anat. **19**: 179, 1916.
(2) EICHELBERGER, L. AND W. G. BIBLER. J. Biol. Chem. **132**: 645, 1940.
(3) WHIPPLE, G. H. This Journal **76**: 693, 1926. –
(4) EICHELBERGER, L. AND M. ROMA. J. Urol. **40**: 366, 1938.
(5) EICHELBERGER, L. J. Biol. Chem. **128**: 137, 1939.
(6) HASTINGS, A. B. AND L. EICHELBERGER. J. Biol. Chem. **117**: 73, 1937.
(7) STANDER, H. J. AND A. W. CREADLICK. Bull. Johns Hopkins Hospital **35**: 1, 1924.
(8) HASTINGS, A. B., H. H. SALVESEN, J. SENDROY, JR. AND D. D. VAN SLYKE. J. Gen. Physiol. **8**: 701, 1927.
(9) GREENE, C. H., J. L. BOLLMAN, N. M. KEITH AND E. G. WAKEFIELD. J. Biol. Chem. **91**: 203, 1931.
(10) CHESLEY, L. C. AND E. R. CHESLEY. Am. J. Obstet. and Gynecol. **42**: 976, 1941.

PENETRATION OF RADIOACTIVE POTASSIUM IN DENERVATED MUSCLE

CHARLES P. LYMAN

From the Department of Physiology in the Harvard Medical School

It is well established that denervation of skeletal muscle causes a sensitization to various drugs which increases for about a week and remains until the nerve regenerates. Although the effect of acetylcholine on denervated muscle has been the chief object of study in this respect, Dale and Gasser (1926) have shown that potassium chloride, nicotine and various other substances will produce typical contractures.

As an explanation of the phenomenon of sensitization in sympathetic ganglia, Cannon and Rosenblueth (1936) suggested that denervation increased the permeability of the cell membranes. Due to the increased permeability a chemical agent would have readier access to the interior of muscle fibers, for example, and thus produce its effect at lower concentrations than in normal muscle. This view has been criticized by Lee (1939, 1940) who was able to show that in the toad the amount of potassium in excised skeletal muscle varied inversely with the sensitivity. He suggested that altered sensitivity was due to an inherent change of irritability attendant on an altered concentration of a certain ion (K). It is questionable, however, whether the condition found in excised muscle represents that found in the intact animal.

The following experiment was designed to compare the permeability of denervated and normal skeletal muscle to radioactive potassium. As Noonan *et al.* (1941b) have shown, simple transection of the sciatic nerve in rats causes a great increase in the rate of penetration of potassium chloride, presumably due, at least in part, to increased blood flow through relaxed vessels. In order to lure out the circulatory effect it was necessary to denervate the muscle without injuring the vasomotor nerve fibers. Since the gray rami of the abdominal sympathetic chains join the lumbar spinal nerves peripheral to the dorsal root ganglia (see Schäfer, 1900, p. 631) it is possible to leave these rami intact by cutting the dorsal and ventral nerve roots close to the spinal cord within the vertebral canal.

Adult albino rats were anesthetized with nembutal, and the dorsal surface of the fourth, fifth and sixth lumbar vertebrae removed, exposing a portion of the cauda equina. The left dorsal and ventral roots of the respective lumbar nerves were then cut, thus completely denervating the left gastrocnemius muscle (see Green, 1935, p. 130). Although no effort was made to maintain strict aseptic conditions, the animals recovered with no untoward effects.

As tests for the results of this operation on the control of the blood supply 3 rats (nos. 1, 2 and 6) were picked at random from the operated group. The following test was carried out 17 days after the operation in rats 1 and 2, and

14 days after the operation in rat 6. The animals were anesthetized with nembutal and the left sciatic nerve exposed in the region of the thigh. A thermocouple was closely applied to the sole of the paralyzed foot and connected with a delicate galvanometer. Stimulation of the sciatic nerve with faradic current caused a drop in the temperature of the foot, indicating that the nervous control of the blood supply was uninjured. Stimulation of the sciatic nerve of normal rats produced the same result. However, during stimulation, some of the leg muscles of the operated animals (not including the gastrocnemius) underwent weak contractions and the leg muscles of normal animals responded with the usual strong tetanus. In order to rule out the effects of muscle tension on the circulation, 2 other operated animals were sacrificed in the following experiment.

One week after the denervating operation the animals were anesthetized with nembutal and artificial respiration was applied. The test for the decrease of foot temperature on the denervated side was then carried out. After establishing the decrease in temperature brought about by stimulation, curare was injected intravenously. When the sciatic was stimulated the temperature of the foot decreased in the same manner as before, in the absence of any movement by the leg muscles. Normal rats reacted in the same manner under identical treatment. It was therefore established that the denervating operation had caused no injury to the nervous control of the blood vessels of the leg.

In testing the permeability of normal and denervated muscles to radioactive potassium the animals were anesthetized with nembutal and both hind legs skinned to above the knee joint. The skin was then replaced so that the muscles were covered. One or 2 cc. of a 1.3 per cent solution of sodium-free radioactive potassium chloride was injected intraperitoneally, and, after a given period had elapsed, both hind legs were removed above the knee. The gastrocnemius muscle of each leg was quickly dissected and frozen in solid carbon dioxide. Each muscle was cut into small pieces in a frozen condition and ground with sand. The proteins were precipitated with trichloroaecetic acid, and the mixture filtered. The filtrate was evaporated and the radioactivity measured with an electroscope. The results of the experiment are shown in the table. The fifth column refers to the time between the injection of the potassium and the removal of the legs.

Although there is great variation in the amount of radioactive potassium taken up by the muscles, and also a large variation in the proportion of radioactivity in each pair of muscles, it is apparent that within the short period of from 2 to about 9 minutes after injection the denervated gastrocnemii exchanged on the average over twice as much radioactive potassium as the normal muscle.

On the other hand, rats 15 and 16 show that if the radioactive potassium remains in the body for 4 hours there is proportionately less radioactive substance in the denervated than in the normal muscle. Noonan et al. (1941a) have shown that penetration of radioactive potassium into muscle is complete in about 4 hours. Since the exchange of radioactive and inactive potassium was complete in rats 15 and 16 there must have been originally less potassium per

TABLE 1
Penetration of radioactive K in denervated muscle

RAT NO.	SIDE	TIME AFTER OPERATION	AMOUNT IN-JECTED	TIME IN BODY	WEIGHT OF MUSCLE	RADIO-ACTIVITY* IN WHOLE MUSCLE	RADIO-ACTIVITY PER GRAM	RADIO-ACTIVITY PER GRAM L/R
		days	cc.	minutes	grams			
1	L	19	2.0	2.0	0.72	15.4	21.4	2.64
	R				1.11	9.0	8.1	
2	L	19	1.0	2.0	0.70	4.0	5.7	1.42
	R				1.01	4.0	4.0	
3	L	8	2.0	2.5	0.87	4.5	5.2	2.26
	R				1.06	2.5	2.3	
4	L	18	2.0	3.0	0.80	8.8	11.0	2.30
	R				1.61	7.8	4.8	
5	L	16	1.0	3.0	0.79	16.8	21.2	1.42
	R				1.40	20.9	14.9	
6	L	16	1.0	4.0	0.65	15.7	24.2	2.64
	R				1.25	11.5	9.2	
7	L	31	1.0	4.0	0.48	7.0	14.6	3.10
	R				1.56	7.4	4.7	
8	L	17	1.0	4.1	0.61	3.7	6.1	2.03
	R				1.61	4.9	3.0	
9	L	8	2.0	4.5	1.08	23.2	21.5	1.75
	R				1.20	14.7	12.3	
10	L	7	1.0	4.5	1.34	16.9	12.6	1.78
	R				1.71	12.1	7.1	
11	L	11	2.0	5.0	0.94	38.3	40.7	2.32
	R				1.27	22.2	17.5	
12	L	11	1.0	5.0	0.81	17.3	21.3	1.83
	R				1.15	13.4	11.6	
13	L	31	1.0	5.0	0.50	8.6	17.4	1.97
	R				1.76	15.5	8.8	
14	L	18	1.0	8.75	0.50	10.0	20.0	2.86
	R				1.65	11.5	7.0	
Average L/R..								2.16
15	L	13	1.0	243	0.84	32.0	38.0	0.79
	R				0.71	34.0	48.0	
16	L	11	1.0	240	1.11	36.3	33.0	0.85
	R				1.63	63.3	39.0	
Average L/R..								0.82

* In arbitrary units.

gram of tissue in the denervated than in the innervated muscle. This is in agreement with the observations of Hines and Knowlton (1933) and of Lee (1939). Evidently the greatly increased rate of penetration into denervated muscle within the first few minutes after injection occurs in spite of the fact that there is proportionately less potassium within the muscle with which the radioactive potassium can exchange.

Apparently, then, a muscle which has been denervated for more than a week becomes more permeable to K ions. Since denervation also causes an increased sensitivity to potassium, it is possible that the sensitivity is due to the increased permeability.

SUMMARY

One week or more after the operation the rate of penetration of radioactive potassium into the denervated gastrocnemius muscle of rats was found greater than in the control muscle. Since the sensitivity of denervated muscle to potassium increases 3 or more days after denervation it is suggested that the increased sensitivity is a result of the greater permeability.

REFERENCES

CANNON, W. B. AND A. ROSENBLUETH. This Journal 116: 408, 1936.
DALE, H. H. AND H. S. GASSER. J. Pharmacol. and Exper. Therap. 29: 53, 1926.
GREEN, E. C. Trans. Am. Phil. Soc. XXVII, New Series, 1935.
HINES, H. M. AND G. C. KNOWLTON. This Journal 104: 379, 1933.
LEE, L. Y. Chinese J. Physiol. 14: 357, 1939.
           Ibid. 15: 485, 1940.
NOONAN, T. R., W. O. FENN AND L. HAEGE. This Journal 132: 474, 1941a.
           Ibid. 132: 612, 1941b.
SCHÄFER, E. A. Textbook of physiology. Vol. II. Young J. Pentland. Edinburgh and London, 1900.

CAROTID SINUS REFLEXES AND CONVULSIONS

E. GELLHORN,[1] L. YESINICK, M. KESSLER AND H. HAILMAN

From the Departments of Physiology and Psychiatry, College of Medicine, University of Illinois, Chicago

It was shown by Koch (1932) that carotid sinus reflexes have a profound influence on the excitability of the central nervous system. Increase in intrasinusal pressure causes sleep in unanesthetized dogs and fall in pressure leads to signs of a general increase in excitability. Observations by Gellhorn, Darrow and Yesinick (1939) showed the applicability of this phenomenon to the study of convulsions. These authors showed that adrenalin in minute quantities inhibits metrazol convulsions and that this effect depended on the presence of the carotid sinus and depressor nerves.

The present paper reports experiments in which a further attempt was made to study the relationship between the depressor reflexes and convulsions. Two problems were investigated:

1. The effect on convulsions of carotid sinus stimulation induced by alterations in intrasinusal pressure.

2. The effect of convulsions on the carotid sinus pressor reflexes.

METHODS. The experiments were performed on narcotized cats and dogs (chloralose 80–100 mgm./kilo). The convulsions were recorded mechanically from the hind leg by means of two tambours and the blood pressure was recorded from the carotid artery. Metrazol, strychnine, camphor, picrotoxin, coryamyrtin[2], and absinth were used as convulsant drugs. In some experiments it was found useful, in order to insure regularity of the convulsions, to use subconvulsive concentrations of the drug and to apply mild electric shocks to the chest and shoulder region. These shocks produced only local contractions in the animal before the convulsant drug was given, but they were adequate to elicit and maintain generalized convulsive movements after the drug had been administered. This procedure was used particularly in experiments with strychnine and camphor. The experiments were performed in 1939 and recently repeated with an improved technique for the recording of convulsions. The convulsing limbs were attached to the input electrodes of a push-pull amplifier. Any motion of the electrodes at their point of attachment to the skin caused a contact potential which was roughly proportional to the intensity of that motion and, therefore, served as a good indicator of the intensity and frequency of the convulsions under various conditions.

RESULTS. *I. The influence of depressor reflexes on convulsions.* The first group of experiments deals with the effect of clamping of the carotid arteries below the bifurcation on chemically induced convulsions. The results were

uniform showing that clamping of the carotid arteries caused an intensification of the convulsions. This is particularly marked in those experiments in which subconvulsive concentrations of drugs such as metrazol or picrotoxin were given or in which the experiments were performed after overt convulsions had ceased completely. Figure 1 shows three records of such experiments in which the clamping of the carotids caused the convulsions to reappear. The quantitative relations as illustrated in figure 1 are of interest. The first graph shows that about 15 seconds following the ligation of the carotid arteries convulsions appeared and persisted for about 15 seconds after the arteries had been released.
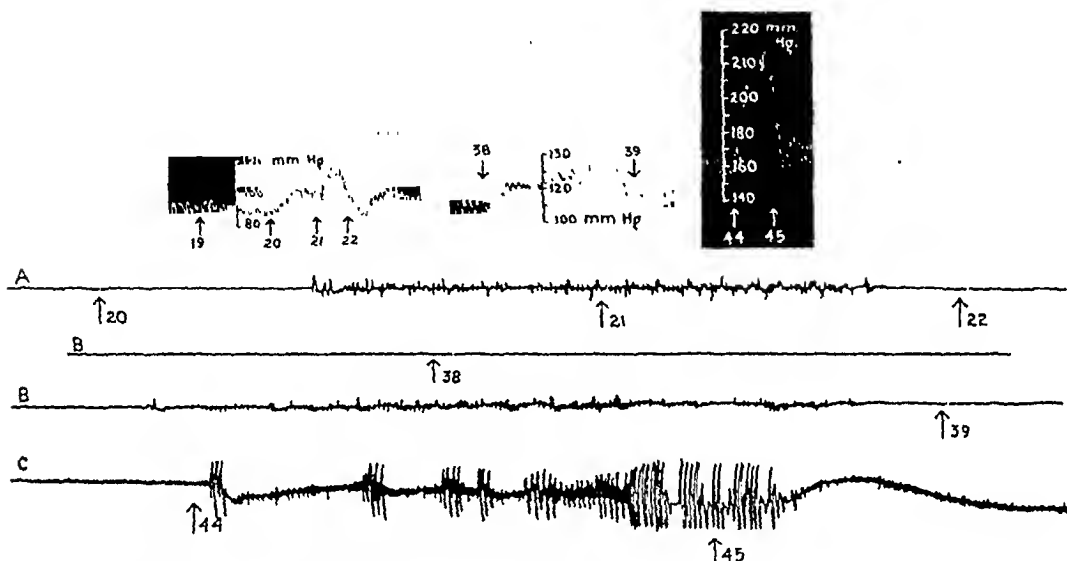
Fig. 1. Effect of clamping of carotids on convulsions. *A* and *B*. Dog, 10 kgm.; chloralose, 80 mgm./kgm. intravenously. Bilateral vagotomy. Blood pressure recorded from femoral artery. Artificial respiration. Convulsions recorded from the hind leg. Convulsions had been elicited three hours earlier by injection of 6 mgm. picrotoxin.

A. 20–21. Both carotid arteries clamped for 30 seconds. *22.* 22 seconds after the release of the carotids. Note after discharge of convulsions.

B. 38–39. Clamping of carotids for 90 seconds. No after discharge of convulsions. The two parts of B in the record of convulsions are continuous.

C. Dog, 7.3 kgm.; operation and procedure as above. *44–45.* Both carotid arteries clamped for 30 seconds. Severe convulsions ceasing a few seconds after release of carotids.

Comparing the blood pressure record with that of the convulsions an interesting parallelism appears between convulsive activity and changes in blood pressure resulting from the clamping of the carotids. In the first blood pressure record of figure 1 it is seen that after the release of the carotids a secondary rise of the blood pressure occurs and that only hereafter the blood pressure falls to its original level. It is at the end of this secondary rise of blood pressure that the convulsions cease abruptly (cf. discussion).

The second graph of figure 1 is obtained from the same animal, sometime later. The carotids are clamped for 90 seconds and convulsions appear after a considerable latent period. They are maintained during the second half of the period

during which the pressure in the carotid sinuses was lowered. The convulsions cease shortly before the clamps are removed (i.e., shortly before no. 39) at a time when the blood pressure begins to fall.

The third record obtained from another animal under similar conditions is characterized by a much greater pressor reflex than had been seen in the first and second graph of figure 1. During the period of clamping convulsions of great intensity occur. As the blood pressure falls abruptly in a few seconds after the removing of the clamps (no. 45) the convulsions disappear.

In other experiments in which convulsions were present prior to the ligation of the carotids the convulsions were increased in intensity during the time of the rise in blood pressure resulting from the clamping of the carotids. The experiments indicate a parallelism between the intensity and duration of sympathetic
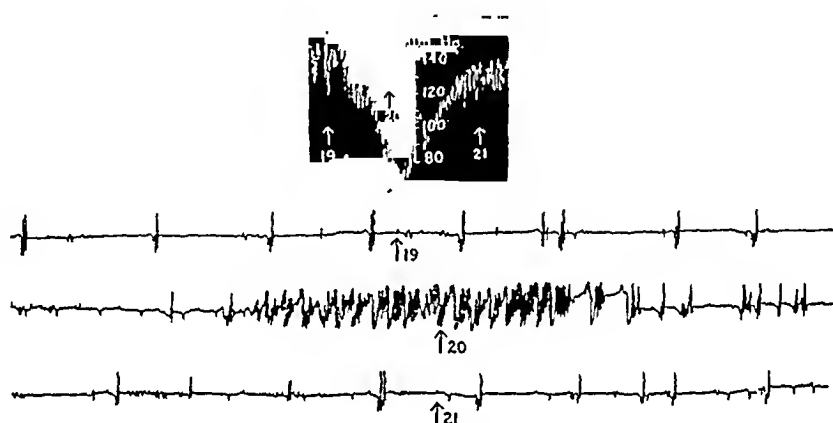
Fig. 2. Effect of amyl nitrite on convulsions. *D*. Dog, 4 kgm.; Dial in urethane 0.5 cc/kgm. Blood pressure recorded from carotid artery. Artificial respiration. Convulsions recorded from the hind leg. Strychnine 0.1 mgm./kgm. intravenously. Mechanical stimulation of the chest by a pendulum. *19–20*, inhalation of amyl nitrite for 45 seconds. *21*, 1 min. later. Note maximal convulsions occurring with maximum fall in blood pressure.

impulses as shown by the rise in blood pressure following the clamping of the carotids and the intensity of convulsions.

If it is correct, as the experiments described in figure 1 suggest, that a fall in blood pressure in the carotid sinus gives rise to central sympathetic discharges which greatly intensify convulsions, it is probable that other factors leading to a fall in pressure in the carotid arteries will likewise augment the intensity of convulsions. This is illustrated in figure 2 in which the fall in blood pressure elicited by inhalation of amyl nitrite called forth very intensive convulsions. It is significant that during the first phase of moderate fall in blood pressure the convulsions remained unchanged. Only on further fall in blood pressure are the convulsions greatly aggravated. When the administration of amyl nitrite is discontinued the convulsions remain augmented as long as the fall in blood pressure continues. Then they return rapidly to the original level.

On the basis of the experiments described thus far and in view of the work of Gellhorn, Darrow and Yesinick, it was thought probable that alteration in pos-

ture leading to variations in intrasinusal pressure may alter the intensity of the convulsions through the depressor reflexes. Figure 3 shows two records demonstrating the influence of increased pressure in the carotid sinus on convulsions. In the first record a cat showing strychnine convulsions was tilted into a head down position of 45° for 15 seconds. The convulsion decreased in intensity almost instantaneously. When the cat was returned to the horizontal position they increased slightly and showed two minutes later the same intensity as was observed prior to the tilting. The carotid pressure rose 20 mm. during the tilting period. In the second experiment of figure 3 the cat was tilted into the same position for 30 seconds. The convulsions decreased gradually in intensity during the tilting and ceased completely at the end of the 30 second period. One and a

Fig. 3. Effect of tilting into head down position on convulsions. Cat, 3 kgm.; Dial in urethane 0.5 cc./kgm. intraperitoneally; artificial respiration. Blood pressure recorded from the carotid artery. Convulsions recorded from the hind legs. Strychnine 0.2 mgm./kgm. intravenously.

A. *3–4*, cat tilted into head down position, angle 45° for 15 seconds. *5*, 2 minutes after return to horizontal position. Blood pressure before *3*, 102 mm. Hg, between *3* and *4*, 122 mm. Hg, and at *5*, 100 mm. Hg.

B. Same animal. *15–16*, cat tilted into head down position, angle 45° for 30 seconds. *16a*, 1½ minutes later and *17*, 5 minutes later. B. P. before *15*, 119 mm. Hg, between *15* and *16*, 138 mm. Hg, and at *17*, 114 mm. Hg.

half minutes later (no. 16a) the convulsions were still markedly depressed but after five more minutes (no. 17) had returned to approximately the original level. Similar results were obtained in numerous experiments on cats and dogs subjected to various convulsant drugs.

A series of experiments was performed in which cats and dogs injected with metrazol, picrotoxin and other convulsant drugs were brought into the opposite position ("feet down" at an angle of 45°). The changes produced in the convulsions under these conditions were only slight or absent. Whenever changes were observed they were opposite to those obtained on tilting into the head down position.

The effect on convulsions of tilting the animal into the "head down" or "feet down" position, the effect of amyl nitrite and of clamping of the carotid arteries

was tentatively related in the previously described experiments to the action of these procedures on the depressor reflexes of the sino-aortic areas. It was, therefore, necessary to study the influence of these factors on convulsions in animals in which the vagi had been cut bilaterally and the carotid sinuses had been denervated.

Figure 4a shows that in such animals tilting into the head down position had an effect opposite to that obtained in animals with normal pressure reflexes. The picrotoxin convulsions which had disappeared prior to the tilting reappeared when the dog was tilted into the head down position for 45 seconds. The effect was reversible when the animal was returned to the horizontal level. In other experiments similar effects were obtained although of varying intensity. Tilting
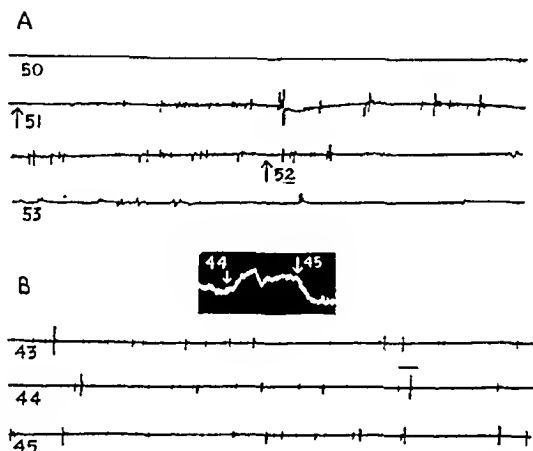
Fig. 4. Effect of tilting and of clamping of carotid arteries on picrotoxin convulsions in a dog deprived of the vagi and carotid sinuses. Dog, 8 kgm.; chloralose 80 mgm./kgm. intravenously. Blood pressure recorded from the femoral artery. Bilateral carotid sinus denervation and vagotomy. Convulsions recorded from hind leg. Picrotoxin 0.3 cc. 0.3 per cent.

A. *50*, animal in horizontal position; no convulsions. *51*, Animal tilted 45°, head down for 45 sec. Note appearance of convulsions. *52*, return to horizontal position. *53*, same condition as in *52*.

B. Same animal. *43*, control; *44*, clamping of carotid arteries. No change in convulsions. *45*, release of carotid arteries. Records *43* to *45* and *50* to *53* are continuous.

the animal into the feet down position caused in some experiments decrease or disappearance of the convulsions particularly when the fall in blood pressure was great. In other instances no changes were observed. Clamping the carotids caused no significant changes in intensity and frequency of the convulsions in animals deprived of the carotid sinus reflexes (fig. 4b). Experiments of the type shown in figure 4b are particularly significant since very slight convulsions are greatly aggravated by clamping of the carotid arteries when the pressure receptors are intact.

The observations indicate that after elimination of the pressure receptors of the sino-aortic areas procedures which in the intact animal modify the intensity of convulsions have either no influence on them or show a reversal of the usual effects.

II. The influence of convulsions on pressure reflexes. The experiments described thus far indicate that the depressor reflexes have a powerful influence on the intensity of convulsions. In view of these facts, it seems to be of interest to investigate whether under conditions of convulsions induced by various convulsant drugs, the intensity of the carotid sinus reflexes is altered. For these purposes, experiments were performed on the influence of tilting on the blood pressure before and during convulsions. A typical experiment is shown in figure 5 from which it is evident that the blood pressure change on tilting into the "head down" as well as into the "feet down" position is greatly reduced in the convulsant animal. Whereas the tilting in the "head down" position caused a blood pressure rise of about 20 mm. in the non-convulsant animal, the blood pressure remained practically unchanged during the metrazol convulsions in the same posture. The tilting into the "feet down" position caused a fall in blood pressure which was maintained as long as this position existed, but the same change in position did not cause any maintained fall in blood pressure in the convulsant animal. The blood pressure fell only for a short period of time, then rose to the initial level. Such fall and rise of blood pressure is seen repeat-

Fig. 5. Cat, 2.2 kgm. Blood pressure from the carotid artery. *5–6*, head down (angle 45°) for 60 sec. *7–8*, feet down (angle 45°) for 45 sec. Convulsions induced by metrazol (0.12 gram intravenously). *13–14*, head down; *15–16*, feet down, as above.

edly in the feet down position. In other experiments it was found frequently that the blood pressure fall on tilting into the feet down position amounted only to 25 or 33 per cent of the fall seen in the same position before the convulsant drug was injected or after the convulsions had ceased.

These experiments suggest that during the convulsions the blood pressure regulating reflexes are intensified.

Discussion. The experiments reported in this paper show that conditions leading to increased sympathetic discharges following a decrease in pressure in the sino-aortic area increase the intensity of existing convulsions or cause convulsions to reappear which had ceased spontaneously. It is interesting to note that the blood pressure change itself is not the all important factor but the pressure in the carotid sinus. This is evident from the fact that amyl nitrite which causes the systemic blood pressure to fall as well as clamping the carotids intensifies convulsions although the blood pressure rises in the latter case. Obviously, the increased depressor discharges originating in the arch of the aorta as the result of the raised systemic pressure following the clamping of the carotids cannot offset the effect which the greatly lowered pressure has on the receptors in the carotid sinus.

How effective this increased sympathetic discharge is was seen in experiments

in which *repeated* clamping of the carotids caused not only a reappearance of convulsions during the periods of lowered carotid sinus pressure but also a persistence of these convulsions for many minutes. Furthermore, the first record of figure 1 shows an after discharge of the convulsions which persists for about 10 seconds after the release of the carotid arteries. During this time the systemic blood pressure shows a secondary rise in spite of a rise in intrasinusal pressure resulting from the release of the carotids. This suggests that increased sympathetic after discharges are responsible for the persistence of the autonomic changes (rise in blood pressure) as well as for the continuation of increased discharges in the somatic nervous system (convulsions). The autonomic afterdischarges are apparently related to the intensification of the carotid sinus reflexes which occurs during convulsions.

In an earlier study (1939) it was shown that adrenalin inhibits convulsions in the normal animal but fails to show this effect after denervation of the sinoaortic area. Similarly, it was shown in the present investigation that the typical effects on convulsions resulting from tilting, amyl nitrite, and clamping the carotids were either absent or reversed after bilateral vagotomy and denervation of the carotid sinuses. Either the vagi or the carotid sinuses were sufficient to regulate the intensity of the convulsions indicating that the pressure receptors of the carotid sinuses as well as those of the aortic arch are involved in this reaction.

The fact that tilting into the "head down" position increases the intensity of convulsions when the pressure receptors had been eliminated but diminishes or abolishes convulsions in the normal animal suggests that the effects on them in the normal animal are not due to changes in brain circulation. Since in animals without pressure receptors tilting into the "head down" position augments convulsions it must be concluded that increased cerebral circulation itself intensifies convulsions, but this effect is overcompensated by the inhibitory impulses originating in the pressure receptors. The earlier studies of Gellhorn, Darrow and Yesinick (1939) had led to similar conclusions.

It will be the task of further investigations to determine where and how the pressure receptors influence brain potentials of convulsant animals.

CONCLUSIONS

The effect of tilting, clamping the carotids and amyl nitrite on convulsions is determined in anesthetized cats and dogs before and after elimination of the pressure receptors. It was found:

1. Lowering of the carotid sinus pressure by clamping the carotids, tilting into the "feet down" position and amyl nitrite lead to an intensification of existing convulsions and to the reappearance of convulsions which had ceased for some time.

2. Raising the pressure in the carotid sinus by tilting the animal into the "head down" position abolishes or reduces convulsions.

3. The effects described under 1 and 2 are absent or reversed after bilateral vagotomy and denervation of the carotid sinuses.

4. The depressor reflexes are intensified during convulsions, since postural

changes produce smaller blood pressure changes in the convulsant than in the non-convulsant animal.

REFERENCES

GELLHORN, E., C. W. DARROW AND L. YESINICK. Arch. Neurol. Psychiat. 42: 826, 1939; Proc. Inst. Med. Chicago 13: 121, 1940.
KOCH, E. Klin. Wchnschr. 11: 225, 1932.

THE SIGNIFICANCE OF CAROTID SINUS REFLEXES FOR THE EFFECTS OF ANOXIA AND CARBON DIOXIDE ON CONVULSIONS[1]

E. GELLHORN and L. YESINICK

From the Departments of Physiology and Psychiatry, College of Medicine, University of Illinois, Chicago

Our previous investigations (1939–40) have shown that the reflexes of the carotid sinus have a profound influence on convulsions induced by various drugs. Since it is known that blood gases play an important part in the genesis of epileptic convulsions (Gibbs, Lennox and Gibbs, 1940) an investigation of the effect of anoxia and carbon dioxide with emphasis on the rôle played by the buffer nerves seemed of importance.

METHOD. The experiments were performed on cats in chloralose narcosis (100 mgm./kgm., subcutaneously). The convulsive movements were recorded from the hind leg through two tambours, and the blood pressure from the carotid artery.

Metrazol, picrotoxin, coryamyrtin,[2] absynthe and strychnine were used as convulsant drugs. Gas mixtures were prepared by flow meters and inhaled from Douglas bags (Gellhorn and Packer, 1940).

RESULTS. Figure 1 shows the effect of anoxia and of carbon dioxide inhalation on convulsions induced by absinthe. It is seen that the inhalation of 7 per cent oxygen for 3 minutes does not alter in any way the convulsions. On the other hand inhalation of 15 per cent carbon dioxide very quickly diminishes the convulsions. The effect is reversible. It is noteworthy that similar effects may be obtained with carbon dioxide when inhaled in much smaller concentrations (5 per cent). A similar result is seen in figure 2 in which weak condenser discharges applied to the shoulder resulted in general convulsive movements in a cat narcotized with chloralose. Here again it was found that the inhalation of an oxygen-nitrogen gas mixture did not influence the convulsive movements, whereas carbon dioxide abolished them completely during the period of inhalation.

Similar results were obtained with the other convulsant drugs listed above. It was invariably found that gas mixtures low in oxygen had little or no effect on convulsions in narcotized cats independent of the nature of the convulsant drug involved. On the other hand carbon dioxide abolished the convulsions reversibly although it was administered for shorter periods than were the anoxic gas mixtures.

The most interesting result of the investigation is the fact that the removal

of the carotid sinuses and the sectioning of the vagi reversed this result. Figure 3 shows that the inhalation of 6.2 per cent oxygen for 3 minutes was without influence on the strychnine convulsions as long as the buffer nerves were intact. If, however, the carotid sinuses were denervated, and the vagi cut, the same gas mixture abolished the convulsions completely when administered for one minute only. Whereas figure 3 shows that the denervation of the carotid sinuses and the sectioning of the vagi greatly increased the effect of anoxia on convulsions, figure 4 indicates that this procedure produces the opposite result with respect to the effect of carbon dioxide. In this figure, it is seen that the inhalation of 15 per cent of carbon dioxide in oxygen abolishes completely coriamyrtin convulsions in the normal cat when administered for 1 minute. After elimination of the buffer mechanisms this gas mixture was found to be ineffective and even 50 per cent CO₂ in oxygen had no influence on the convulsions although it was administered for a longer period of time.
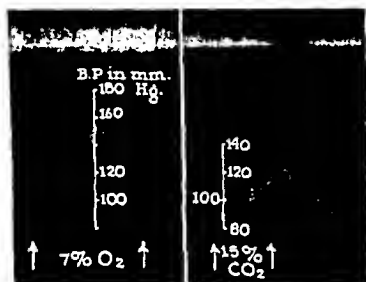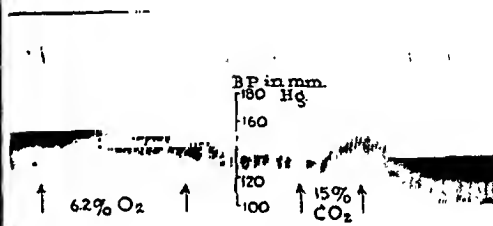
Fig. 1   Fig. 2

Fig. 1. Cat 2.3 kgm, chloralosane 100 mgm./kgm. subcutaneously. Five-tenths cubic centimeter absinthe intramuscularly and 0.5 cc. intravenously. Condenser discharges applied to shoulder. Convulsions recorded from right hind leg (top curve); middle curve: blood pressure. Seven per cent O₂ for 3 minutes between the signs. Fifteen per cent CO₂ for 1.5 minutes between the signs.

Fig. 2. Cat 2.6 kgm., chloralosane 100 mgm./kgm. subcutaneously. Condenser discharges applied to shoulder. Effect of 6.2 per cent O₂ and 15 per cent CO₂ in O₂ respectively on chloralosane convulsions.

Figure 5 shows the effect of anoxia and of carbon dioxide on metrazol convulsions in a cat in which the buffer nerves have been cut. Fifteen per cent carbon dioxide in oxygen administered for 3½ minutes abolished temporarily the convulsions, but the administration of 6.2 per cent oxygen for 1 minute only, caused an inhibition of the convulsions for a much longer period of time. Whereas in the normal cat metrazol convulsions are easily abolished by carbon dioxide and anoxia has very little if any influence on the convulsions the opposite is true after the carotid sinus nerves and the vagi have been sectioned.

Figure 6 illustrates the effect of carbon dioxide and anoxia on camphor convulsions. Carbon dioxide does not interfere at all with these convulsions in the "denervated" animal, whereas a relatively mild anoxia (7 per cent oxygen) abolishes them completely. The convulsions reappear, however, in full strength, about 2 minutes after air has been readmitted.

The fundamental difference in the reaction of convulsing animals to carbon

dioxide and anoxia can also be shown if, instead of permitting the animal to inhale gas mixtures low in oxygen, asphyxia is induced by clamping of the trachea. Figure 7 shows that the inhalation of 15 per cent carbon dioxide inhaled for 3 minutes does not alter the convulsions produced by picrotoxin in a "denervated" animal. When, however, the trachea was clamped for 1 minute the
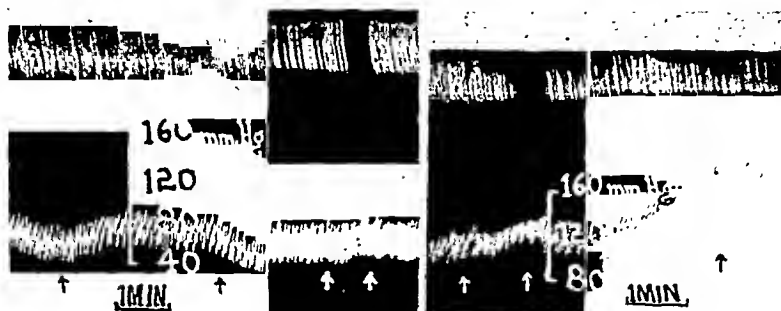
Fig. 3 Fig. 4

Fig. 3. Cat 2.6 kgm., chloralosane 100 mgm./kgm. subcutaneously. Twenty-five hundredths strychnine (1:1000) intravenously and 0.13 strychnine intramuscularly. Condenser discharges as in figure 1. Effect of inhalation of 6.2 per cent O_2 on strychnine convulsions before (left figure) and after denervation of carotid sinuses and bilateral vagotomy (right figure)-

Fig. 4. Left figure. Cat 2.5 kgm. chloralosane 100 mgm./kgm. subcutaneously. Twenty-five hundredths milligram coriamyrtin intravenously. Between the arrows 15 per cent CO_2 in oxygen is inhaled.

Right figure. Cat 2.2 kgm. One hundred milligrams chloralosane subcutaneously; 0.2 mgm. coriamyrtin intravenously. Bilateral vagotomy and denervation of the carotid sinuses. Between the arrows 50 per cent CO_2 in oxygen is inhaled.

Fig. 5. Cat, 2.6 kgm., chloralosane 100 mgm./kgm. subcutaneously; 0.25 cc. 10 per cent metrazol intravenously. Effect of 6.2 per cent O_2 (for 1 min.) and of 15 per cent CO_2 in O_2 for 3.5 minutes on metrazol convulsions in the "denervated" cat (carotid sinuses denervated and vagi cut).

convulsions were almost immediately abolished and returned only after readmission of air and then at greatly reduced intensity.

DISCUSSION. The experiments have shown clearly that both carbon dioxide and anoxia can reversibly abolish chemically induced convulsions. Whereas anoxia is far more effective than carbon dioxide in the animal whose buffer nerves had been eliminated it is found that the opposite is true for the animal

with the carotid sinus nerves and vagi intact. It is very unlikely that this result is due to the action of gases low in oxygen and high in carbon dioxide on the chemoreceptors in the carotid sinuses and in the arch of the aorta. First, it is well known that anoxia and carbon dioxide are excitatory stimuli for the chemoreceptors and can therefore not be the cause of the reversal of the effects of these gases resulting from the denervation of the carotid sinuses. Moreover, experiments of Greenberg and Gellhorn (1940) have shown that the effect of carbon dioxide and oxygen lack on medullary reflexes (linguo-maxillary reflex) are not modified by the elimination of the buffer nerves. On the other hand, it is known from our previous investigations that the buffer nerves inhibit convulsions. Since it was shown that the depressor reflexes are involved, it seems most likely that the results reported in this paper are due to a modification of these reflexes under conditions of anoxia and hypercapnia. Gellhorn

Fig 6.

Fig. 7.
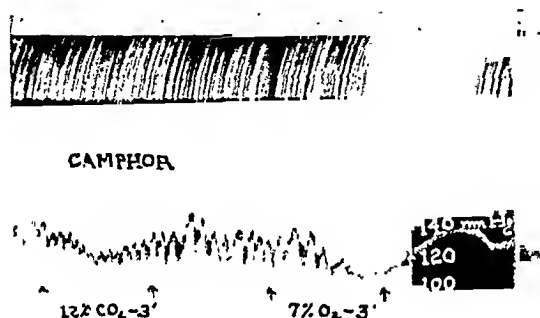
Fig. 6. Cat, 3 kgm., chloralosane 100 mgm./kgm. subcutaneously. Five cubic centimeters 20 per cent camphor in oil subcutaneously. Condenser discharges applied to the shoulder. Carotid sinuses denervated and vagi cut. Between the arrows 12 per cent CO₂ and 7 per cent O₂ respectively for 3 minutes.
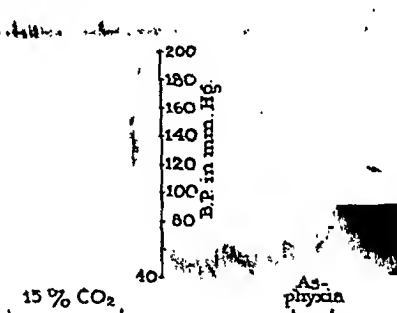
Fig. 7. Cat 2.3 kgm. chloralosane 100 mgm./kgm. subcutaneously. Carotid sinuses denervated, vagi cut. Five-tenths cubic centimeter picrotoxin 1:1000 intravenously. Between 9 and 10, 15 per cent CO₂ in O₂ for 3 minutes. Between 11 and 12, artificial respiration stopped for 1 minute and trachea clamped.

and Lambert (1938, 1939) have shown that the reflexes of the carotid sinuses are weakened by anoxia. Van der Linden (1933) and Gellhorn and Pollack have found that during inhalation of carbon dioxide, the depressor reflexes are intensified. From the fact that anoxia exerts a strong inhibitory effect on the convulsions in the "denervated"[3] animal whereas carbon dioxide is without any effect or only slightly effective, it must be concluded that the excitability of the neurons involved in the production of the convulsions is more susceptible to anoxia than to an increased carbon dioxide tension. These effects are modified by the pressure reflexes originating in the carotid sinuses and the arch of the aorta. The inhibitory action of these reflexes on convulsions is weakened in anoxia because the depressor reflexes are weakened and consequently this effect is antagonistic to the inhibitory action which anoxia exerts on the neurons themselves. On the other hand, the inhibitory action of the pressure reflexes on

---

[3] After denervation of the carotid sinuses and bilateral vagotomy.

convulsions is intensified during carbon dioxide inhalation and consequently leads to the abolishment of convulsions although the effect of carbon dioxide on the brain itself is too weak to produce such a result.

SUMMARY

The influence of anoxia and of inhalation of carbon dioxide is investigated on chemically induced convulsions in the narcotized cat. It is found that anoxia promptly abolishes convulsions in the cat whose carotid sinus nerves and vagi have been cut, whereas it exerts no or a very slight inhibitory effect on the normal animal. On the other hand, carbon dioxide is a powerful inhibitory agent as far as convulsions are concerned in the normal animal, but fails to show such effects after the denervation of the carotid sinuses and bilateral vagotomy. The peculiar reversal in the efficacy of carbon dioxide and anoxia which is produced by the elimination of the buffer nerves is explained by the fact that variations in the tension of oxygen and carbon dioxide in the blood and tissues modify the depressor reflexes originating in the carotid sinus and arch of the aorta in opposite directions.

REFERENCES

GELLHORN, E., C. DARROW AND L. YESINICK. Arch. Neurol. and Psychiat. 42: 826, 1939.
GELLHORN, E., AND E. LAMBERT. Proc. Soc. Exper. Biol. and Med. 38: 315, 1938.
Univ. of Ill. Press, 1939.
GELLHORN, E., AND A. PACKER. This Journal 129: 610, 1940.
GELLHORN, E. AND F. POLLACK. Unpublished observations.
GELLHORN, E., L. YESINICK AND C. DARROW. Proc. Inst. Med. Chicago 13: 121, 1940.
GIBBS, E. L., W. G. LENNOX AND F. A. GIBBS. Arch. Neurol. and Psychiat. 43: 223, 1940.
GREENBERG, R. AND E. GELLHORN. This Journal 129: P 367, 1940.
VAN DER LINDEN, P. Arch. Int. Pharmacodyn. 46: 63, 1933.
YESINICK, L., E. GELLHORN AND C. DARROW. This Journal 129: 502, 1940.

THE TERMINATION OF ASCENDING TRIGEMINAL AND SPINAL TRACTS IN THE THALAMUS ᐧOF THE CAT[1]

H. W. MAGOUN AND W. A. McKINLEY

From the Institute of Neurology, Northwestern University Medical School

In a recent study of the course of a fast-conducting, afferent trigeminal system, McKinley and Magoun (1942) described secondary trigeminal connections from the main sensory and spinal fifth nuclei which passed to the ventro-medial part of the opposite side of the medulla, to ascend toward the thalamus.

Using the same method of study, the oscillographic recording of action potentials evoked by trigeminal nerve stimulation, the present investigation is concerned with the more rostral course of this connection, and with the outcome of its activity at the thalamic level. Because the spatial representation of cutaneous sensibility within the neuraxis is of interest, the distribution of potentials evoked by peripheral stimulation of fore and hindlimb nerves has been compared with those initiated at the face.

METHODS. In cats under nembutal anesthesia fascicles of the infraorbital branch of the trigeminal nerve at the face, the median or ulnar nerves at the wrist, and the superficial peroneal nerve at the ankle, were stimulated with single condenser discharges delivered through a transformer. The action potentials evoked were recorded from the interior of the brain stem with a steel needle electrode, insulated except for 0.1 to 0.2 mm. at the tip, and oriented in the brain with the Horsley-Clarke instrument in the manner described by Ranson (1934). An indifferent electrode was placed in the visual cortex. The remainder of the procedure was similar to that employed by McKinley and Magoun (1942).

RESULTS. *Characteristics of the recorded potentials.* When peripheral nerves of the face or limbs were stimulated and records were taken of the electrical activity in afferent pathways in the upper brain stem, spike-like potentials from fast-conducting elements were obtained from the medial lemniscus, the ventral thalamic nucleus and the internal capsule. At the midbrain level, these spikes commenced 2–2.5 msec. after peripheral trigeminal stimulation, 4–5 msec. after stimulation of the forelimb nerves and 7–9 msec. after stimulation of hindlimb nerves. In the case of potentials recorded from the internal capsule, the latencies following stimulation of face, forelimb and hindlimb nerves were respectively 3–4, 5.5–7 and 9–12 msec. The delay is attributed for the most part to nuclear delay within the thalamus.

The discharge of lemniscal and thalamic neurons may be further distinguished by the more prolonged recovery times of the thalamic neurons, shown by Marshall (1941). In the present experiments, tests with 2 stimuli indicated that the absolutely unresponsive period for face as well as limb responses ranged

between 6 and 18 msec. at the lemniscal level and between 30 and 50 msec. at the level of the internal capsule.

Records shown in figure 1 illustrate the potentials obtained from midbrain (A), thalamus (B) and internal capsule (C) upon stimulation of trigeminal (1), fore-limb (2) and the hindlimb (3) nerves. The increase in latency encountered between the lemniscus and internal capsule is seen, the latencies in msec. to the beginning of the spike in each record being: 1A—2, B—2.2, C—3; 2A—4, B—5.6, C—6.5; 3A—9.5, B—12. It may be noted that in potentials recorded from the thalamus (figs. 1 and 4, 1–3B), the initial positive spike is followed by a negative deflection which is not present in records from the medial lemniscus (fig. 1, 1–3A) or internal capsule (fig. 1, 1–2C). In recording from the ventral thalamic nucleus, the discharge of ramifying terminals of lemniscal fibers and of thalamic axons coursing toward the internal capsule would appear to complicate the dis-
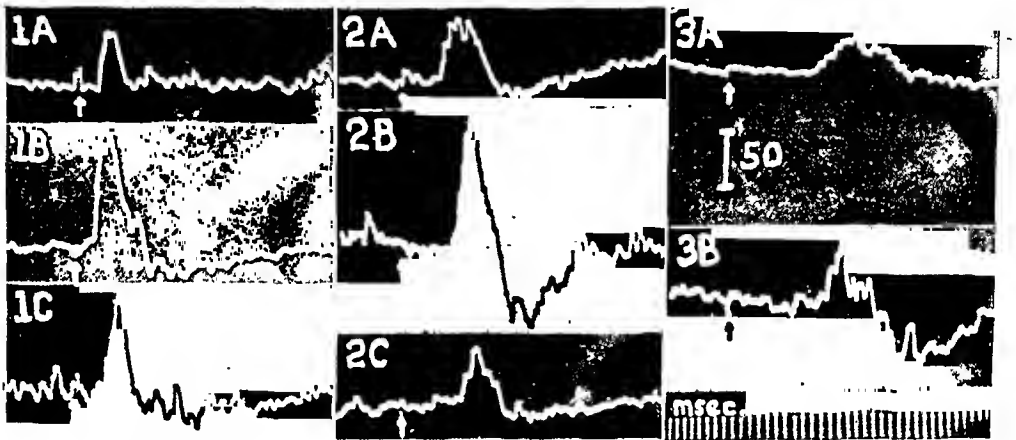
Fig. 1. Action potentials recorded from the medial lemniscus (A), ventral thalamic nucleus (B) and internal capsule (C) upon stimulation of peripheral branches of trigeminal (1), forelimb (2) and hindlimb (3) nerves. Application of maximal shocks shown by arrows, time in msec. and calibration in μV. An upward deflection indicates positivity at the needle electrode.

charge of any particular group of thalamic cells. The potentials recorded from the ventral nucleus in these experiments have usually resembled those from the lemniscus or internal capsule rather than the barrage of diphasic or negative spikes found during a similar time interval by Marshall (1941).

The distribution of points from which these action potentials of fast-conducting afferent neurons have been recorded is shown in figure 2 by solid symbols. Potentials evoked by stimulating peripheral branches of the trigeminal, forelimb or hindlimb nerves on the opposite side of the body are indicated respectively by circles, triangles and squares.

Midbrain. Through the midbrain fast potentials from limbs and face were recorded from the medial lemniscus and, in the case of the face, from the adjacent dorsomedial area as well (fig. 2A). At more anterior mesencephalic levels where the medial lemniscus is located above the substantia nigra or where it occupies a region on the medial aspect of the medial geniculate body, this lemniscal
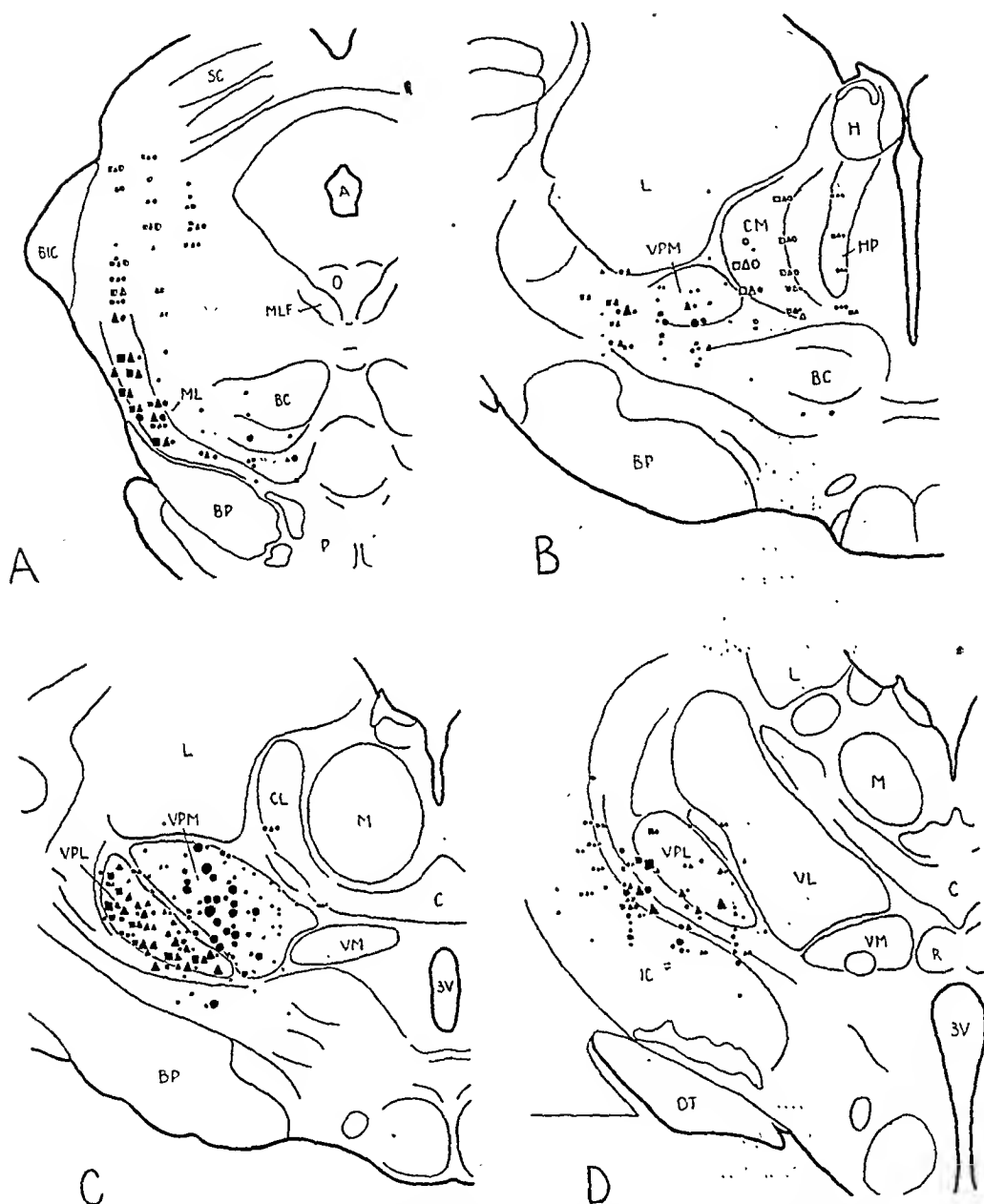
Fig. 2. Transverse sections through the left half of the brain stem of the cat showing the distribution of points from which potentials were recorded upon stimulating peripheral nerves on the opposite side of the body. Potentials of fast-conducting systems are shown with solid symbols: circles for face, triangles for forelimb and squares for hindlimb. Later and more dispersed potentials are shown with half-filled (A) or stippled symbols (B). Abbreviations are as follows:

A, aqueduct; BC, brachium conjunctivum; BIC, brachium of inferior colliculus; BP, basis pedunculi; C, nucleus centralis; CL, nucleus centralis lateralis; CM, centre median; H, habenula; HP, habenulo-peduncular tract; IC, internal capsule; L, nucleus lateralis; LG, lateral geniculate body; M, nucleus medialis; ML, medial lemniscus; MLF, medial longitudinal fasciculus; O, oculomotor nucleus; OT, optic tract; P, pons; R, nucleus reuniens; SC, superior colliculus; VL, nucleus ventralis lateralis; VM, nucleus ventromedialis; VPL, nucleus ventralis posterolateralis (externa); VPM, nucleus ventralis posteromedialis (arcuata); 3V, third ventricle.

distribution of fast-conducting fibers was maintained. It is not known whether the potentials recorded from the mesencephalic course of the medial lemniscus upon stimulation of limb nerves are derived entirely from fibers arising in the posterior column nuclei (Therman, 1941) or whether they are obtained in part also from intermixed fibers of the spinothalamic tracts. In individual experiments a tendency was evident for a mediolateral arrangement of face, forelimb and hindlimb potentials within the lemniscus; this is obscured upon assembling the data from a number of experiments, as is the case at each of the levels in figure 2.

Ventral thalamic nucleus. At the caudal end of the diencephalon where the medial lemniscus is commencing to enter the ventral thalamic nucleus, potentials evoked by trigeminal stimulation are seen to be recorded from its posteromedial part, while those produced by the stimulation of limb nerves are obtained chiefly from more laterally situated points (fig. 2B).

At the level at which the ventral thalamic nucleus reaches its greatest extent, the spatial representation of face and limb potentials in its medial and lateral parts is remarkable (fig. 2C). Hindlimb representation is arranged around the outer rim of the posterolateral nucleus. Forelimb representation is present here as well, and extends through the remainder of the posterolateral nucleus. Trigeminal representation is almost entirely confined to the posteromedial nucleus (fig. 1C). Forelimb representation appears greater than that of the hindlimb, and that of the face appears almost as great as the representation of the limbs combined. Upon stimulating branches of the maxillary division, the largest trigeminal potentials were recorded from the central portion of the posteromedial nucleus (fig. 2C). In instances in which the mandibular or ophthalmic divisions of the trigeminal were employed, their projection was to the same region. Fragmentary data indicate an ipsilateral trigeminal representation in the posteromedial nucleus, perhaps more laterally situated than the contralateral representation.

Farther rostrad (fig. 2D), the posteromedial nucleus is no longer present, but limb afferents are still terminating in the rostral end of the posterolateral nucleus. At this level potentials from axons of more caudally placed thalamic neurons, mediating sensation from the face and limbs, are recorded in the reticular thalamic nucleus and the internal capsule as these axons course toward the cerebral cortex (fig. 2D). At still more anterior levels all are found in the internal capsule.

On a few occasions face and limb potentials similar to those of the lemniscal system were recorded from the subthalamus and zona incerta at the levels of figure 2, B and C. They were obtained from points definitely outside of the external medullary lamina, and appear to indicate the presence of an extrathalamic collateral from the lemniscal pathway.

Protocol. The spatial projection of pathways from the face and limbs upon the ventral thalamic nucleus is as evident in a single experiment as upon assembling the data from a number of animals. In the protocol illustrated in figure 3, punctures 1 to 5 were made at millimeter intervals and in each puncture
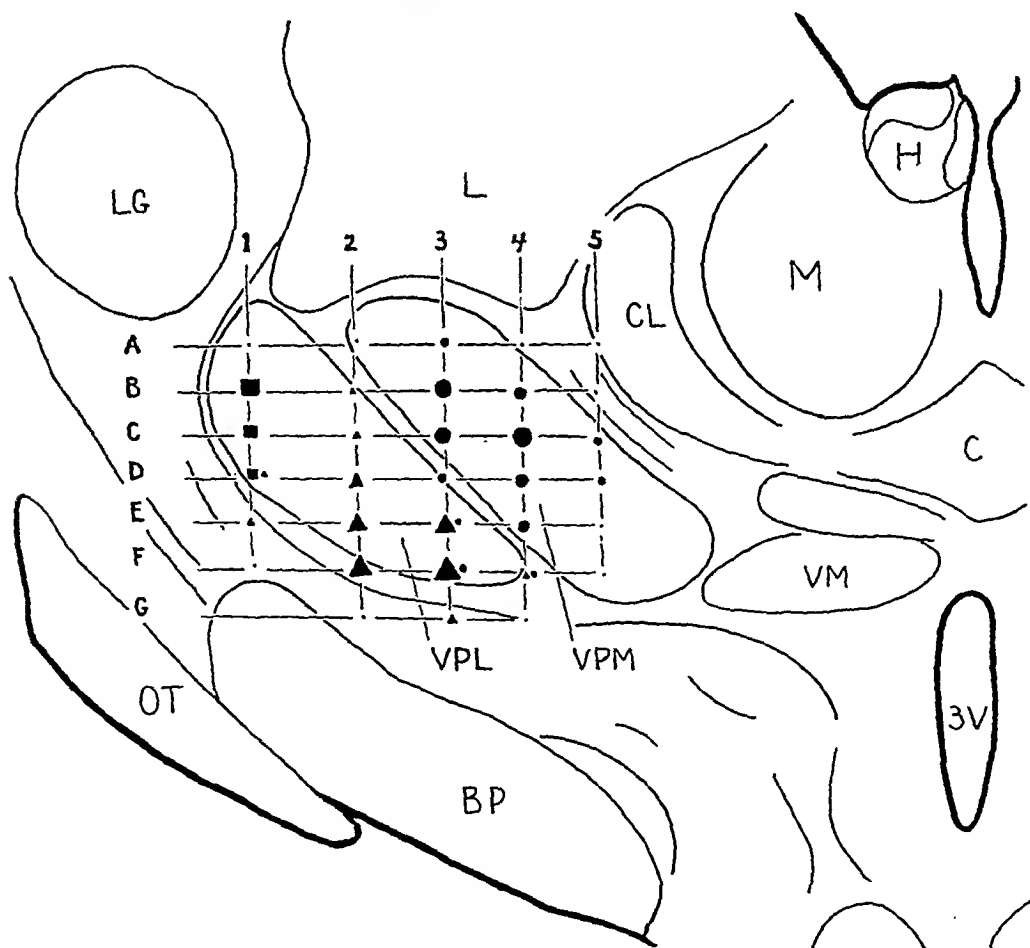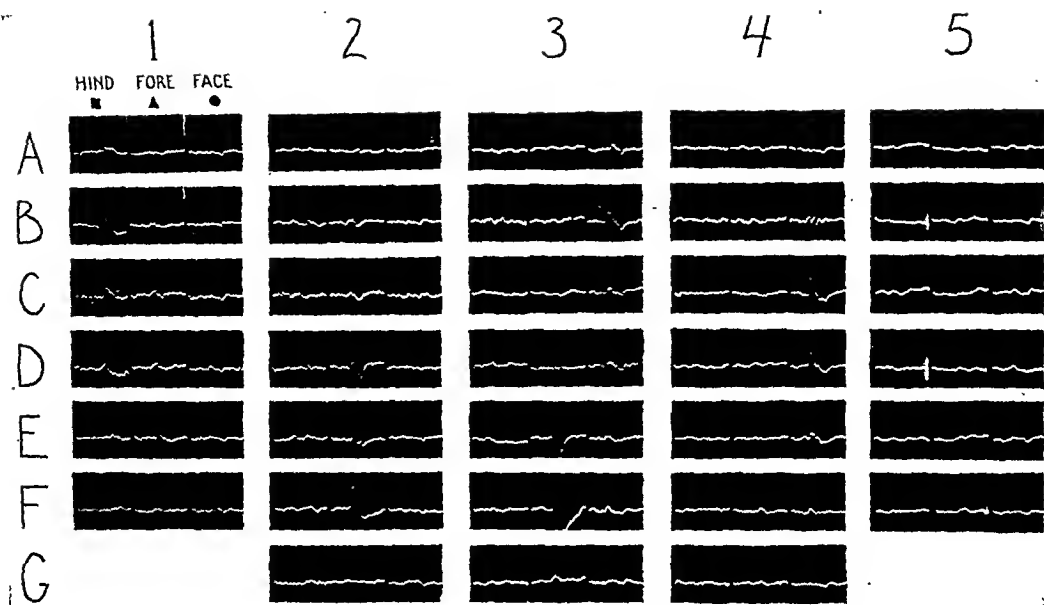
Fig. 3. Protocol of experiment showing potentials evoked by serially stimulating nerves of hindleg, foreleg and face and recording from stops A to G on punctures 1 to 5 through the ventral nucleus of the thalamus.   Symbols and abbreviations as in figure 2.

413

the electrode was halted at half millimeter intervals A to G, and the nerves of the hindleg, foreleg and face on the opposite side of the body were serially stimulated. Hindleg activity was recorded only in puncture 1 at stops B–D in the external part of the posterolateral nucleus. Foreleg activity was obtained at stops B–F in puncture 2 and stops E–G in puncture 3, and was extensively distributed through the medial part of the posterolateral nucleus. Face activity was recorded at stops A–F in puncture 3, stops B–F in puncture 4 and stops C–D in puncture 5, and was distributed through the posteromedial part of the ventral nucleus. From within outward in the ventral nucleus, representation is seen to be in the order of face, forelimb and hindlimb.

Centre median. In the caudal part of the thalamus potentials of a character significantly different from those of the lemniscal system were recorded from points designated by stippled symbols in figure 2B, and distributed in and near
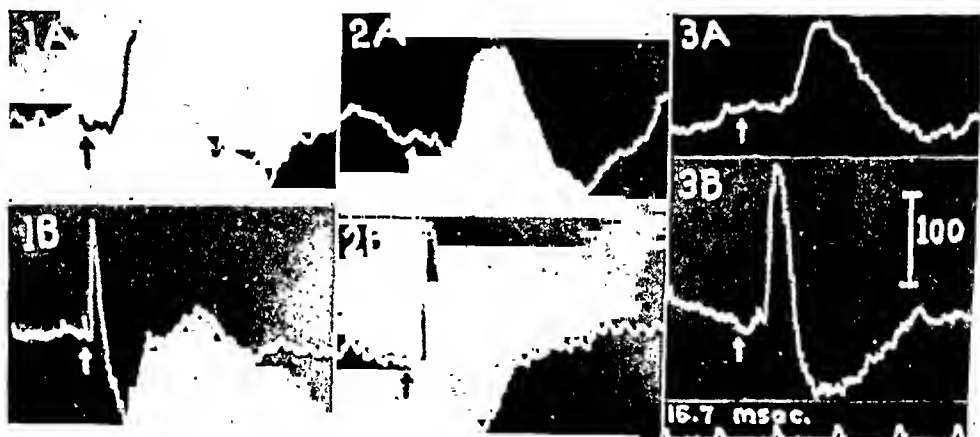
Fig. 4. Potentials recorded from the centre median (A) and from the region where the lemniscus is entering the ventral thalamic nucleus at the same level (B), upon stimulating trigeminal (1), forelimb (2) and hindlimb (3) nerves. Latencies in msec. are: 1 A-9, B-2; 2 A-12, B4.5; 3 A-15, B-7. Calibration in μV. and time in 16.7 msec.

the centre median. Here a late discharge was evoked by peripheral stimulation of both face and limb nerves; the record of this discharge has the form of a wave rather than a spike and was of long duration lasting from 20 to 30 msec. (fig. 4, 1–3A). The latency of these potentials increased with the increased distance of peripheral stimulation but the increment added to the latency of the lemniscal discharge at this level appeared to be a fairly constant one of 7 to 10 msec. In figure 4 the latency and duration of the centre median discharge (fig. 4, 1–3A) is compared with that of the lemniscal-ventral nucleus system at the same level (fig. 4, 1–3B).

If these centre median potentials were elicited from the thalamic termination of slowly conducting afferent pathways, the progressively greater conducting distances from face and limbs should have resulted in increases in latency markedly greater than those found. The threshold of peripheral stimulation required for producing the centre median discharge was low and similar to that required for the discharge of the ventral thalamic nucleus. Both observations suggest

that the late centre median discharge is the result of activity delayed in some relay system. Whether of significance or not this activity was best obtained in 2 animals with chronic lesions of the sensory cortex and extensive though not complete degeneration of the ventral thalamic nucleus.

Evoked potentials intermediate in character between those of the lemniscal system and those of the centre median were sometimes encountered in the region of the mesencephalic course of the spinothalamic tracts, upon stimulation of face as well as limb nerves (fig. 2A, half-filled symbols). These potentials were of small magnitude and long duration, up to 10 or 12 msec. Their latency was 2 or 3 msec. greater than that of the lemniscal spikes. The threshold for obtaining them was low and similar to that required for the lemniscal discharge.

DISCUSSION. Of the ascending pathways to the thalamus, those mediating sensation from the oral cavity and face have been least well worked out. Practically nothing is known concerning the ascending course and thalamic termination of the gustatory lemniscus. More attention has been devoted to afferent trigeminal pathways, and the recent degeneration study by Walker (1939) in the monkey has demonstrated crossed secondary trigeminal pathways passing forward in the spinothalamic tracts and in and adjacent to the medial lemniscus to terminate in the posteromedial part of the ventral thalamic nucleus. The present results on the cat are in general agreement with Walker's observations.

The results of the present study and those of Ranson and Ingram (1932) on the termination of the medial lemniscus and brachium conjunctivum, present a picture of the functional subdivision of the ventral thalamic nucleus of the cat, based on the termination of afferent systems within it, which appears identical with that formulated by Waller (1940) on the basis of retrograde degeneration studies following lesions of the sensory cortex. These results distinguish the terminal regions for the trigeminal lemniscus and the brachium conjunctivum, but otherwise support the topographic analysis of the thalamus of the cat presented by Ingram, Hannett and Ranson (1932).

No study has been made of the modality of sensation transmitted by the lemniscus-ventral thalamic nucleus pathway investigated in these experiments. By analogy with the results of Marshall (1941) and Harrison and Corbin (1942) it is likely that it mediates tactile sensation. The latency of potentials encountered at the level of the internal capsule suggests that the discharge of this system accounts in part at least for the "primary response" of the sensory cortex to peripheral stimulation, which Dempsey, Morison and Morison (1941) have abolished by subcortical lesions in a position to interrupt this pathway, and which Morison, Dempsey and Morison (1941) and Morison and Dempsey (1942) have evoked by direct stimulation of the ventral thalamic nucleus. Indications of activity in the centre median following peripheral nerve stimulation, and presumably induced through some relay system, are of interest because of the distribution of this activity in the caudal part of the intralaminar thalamic area the stimulation of which by Morison and Dempsey (1942) and Dempsey and Morison (1942) has resulted in a "recruiting response" over an extensive region of the cerebral cortex.

SUMMARY

With oscillographic recording of potentials evoked by peripheral nerve stimulation, the mesencephalic course and thalamic termination of fast-conducting pathways from face and limbs have been studied in the cat.

These pathways ascend in and adjacent to the medial lemniscus and terminate in the ventral thalamic nucleus, the limb pathways in its posterolateral or external division and the trigeminal pathways in its posteromedial or arcuate division.

The discharge of these pathways fires cells of the ventral thalamic nucleus whose axons pass forward in the internal capsule toward the cortex. Apparently by some·relay system the centre median is also fired, but considerably later. No evoked potentials were detected in other thalamic nuclei.

REFERENCES

DEMPSEY, E. W., R. S. MORISON AND B. R. MORISON. This Journal **131**: 718, 1941.

DEMPSEY, E. W. AND R. S. MORISON. This Journal **135**: 293, 301, 1942.

HARRISON, F. AND K. B. CORBIN. Anat. Rec. **82**: 26, 1942.

INGRAM, W. R., F. HANNETT AND S. W. RANSON. J. Comp. Neurol. **55**: 333, 1932.

MARSHALL, W. H. J. Neurophysiol. **4**: 25, 1941.

McKINLEY, W. A. AND H. W. MAGOUN. This Journal **137**: 217, 1942.

MORISON, R. S., E. W. DEMPSEY AND B. R. MORISON. This Journal **131**: 732, 1941.

MORISON, R. S. AND E. W. DEMPSEY. This Journal **135**: 281, 1942.

RANSON, S. W. Psychiat. neurol. Bl., Amst. **38**: 534, 1934.

RANSON, S. W. AND W. R. INGRAM.· J. Comp. Neurol. **56**: 257, 1932.

THERMAN, P. O. J. Neurophysiol. **4**: 153, 1941.

WALKER, E. A. J. Comp. Neurol. **71**: 59, 1939.

WALLER, W. H. J. Comp. Neurol. **73**: 117, 1940.

THE EFFECT OF UROGASTRONE ON GASTRIC SECRETION IN ENTERECTOMIZED DOGS[*]

M. J. SCHIFFRIN[1] AND JOHN S. GRAY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Both urogastrone, a substance extracted from the urine, and enterogastrone, a hormone liberated by the small intestine, inhibit gastric secretion. The possibility arose that urogastrone acted through the liberation of enterogastrone because of the similarity of some of their physiological characteristics. If such were the case, then the presence of the small intestine, which is the source of enterogastrone, would be necessary for the inhibition of gastric secretion by urogastrone. Friedman (1941) reported that a gastric secretory depressant, extracted from urine, was ineffective after the removal of the small intestine in sacrifice experiments on dogs. Unpublished data obtained in this laboratory, however, indicated that crude preparations of urogastrone were effective inhibitors of gastric secretion in the absence of the small intestine. Because of this apparent discrepancy, it was thought worthwhile to investigate this problem using more highly purified extracts of urogastrone and chronic enterectomized dogs.

METHODS. In order to study the effect of urogastrone on gastric secretion in enterectomized animals, the entire small intestine from the pylorus to the ileocecal sphincter was removed under aseptic conditions in 6 dogs. The body of the pancreas was excised, the common bile duct ligated, and external drainage of the stomach was established with the aid of a Pezzer catheter. The head and tail of the pancreas, with their blood supplies intact, were tied so as to prevent drainage of pancreatic juice into the abdominal cavity. The dogs were maintained postoperatively by the subcutaneous administration of an aqueous solution containing 0.8 per cent NaCl, 0.03 per cent KCl, 0.014 per cent $CaCl_2$, and 0.016 per cent $MgCl_2 \cdot 6H_2O$ at 6-hour intervals. Eight dogs with vagotomized total gastric pouches were used as controls.

Experimentation was begun two days after the operation as follows: Gastric secretion was stimulated by the subcutaneous injection of 0.15 mgm. histamine dihydrochloride at 10-minute intervals and was collected in 20-minute periods. When the rate of secretion became constant for three consecutive periods, 1.0 mgm. urogastrone was injected intravenously, and the administration of histamine was continued for an additional two hours' time. The free acidity of the gastric juice was determined by titration with standard alkali with Töpfer's reagent as the indicator. The urogastrone used in this study assayed 0.25 mgm. per "dose" (Gray, Wieczorowski, Wells and Harris, 1942).

RESULTS. Eight control experiments were performed on eight dogs with vagotomized total gastric pouches. In every experiment the volume of secretion

and the output of free acid were decreased during the first hour following the injection of urogastrone. The average volume of secretion during the first hour was 92.3 cc. and the average output of free acid was 11.260 m. eq. During the hour following the injection of urogastrone, the average volume of secretion was reduced to 51.5 cc. and the average output of free acid to 6.638 m. eq. (table 1).

Twelve similar experiments were performed on 6 dogs in whom the entire small intestine had been removed. The data of this series are shown in table 1. The volume of secretion and the output of free acid during the hour following the injection of urogastrone were always inhibited in a manner similar to that ob-

TABLE 1

The effect of urogastrone on gastric secretion in enterectomized dogs

DOG NO.	DATE	1ST HOUR*		2ND HOUR		3RD HOUR	
		Vol.	Output of free acid	Vol.	Output of free acid	Vol.	Output of free acid
		cc.	m. eq.	cc.	m. eq.	cc.	m. eq.
5	10/22/41	24	1.530	19	0.826	18	1.040
5	10/23/41	36	2.786	28	1.472	40	2.380
6	10/22/41	51	6.054	28	2.896	37	4.066
6	10/23/41	64	8.494	21	2.268	41	3.210
8	10/27/41	101	12.534	70	8.408	82	10.552
9	11/12/41	149	16.662	69	7.336	97	10.870
9	11/13/41	123	12.702	53	4.156	64	5.620
9	11/14/41	97	10.382	38	2.320	39	1.748
9	11/15/41	157	16.776	59	3.398	39	2.916
13	11/28/41	97	7.438	23	1.166	11	0.212
14	11/28/41	59	6.126	37	3.264	52	5.378
14	11/29/41	91	8.722	30	1.816	34	1.532
Average†		87.4	9.184	39.6	3.277	46.2	4.127
Average of controls‡....		92.3	11.260	51.5	6.638	71.3	8.435

* After first hour, 1.0 mgm. urogastrone injected intravenously.
† Average of 12 experiments on 6 enterectomized dogs.
‡ Average of 8 experiments on 8 dogs with vagotomized total gastric pouches.

served in the total gastric pouch dogs. The average volume of secretion was reduced from 87.4 to 39.6 cc., and the average output of free acid from 9.184 to 3.277 m. eq.

In order to make a comparison of the effect of urogastrone on gastric secretion in the control and enterectomized dogs, the volume of secretion and output of free acid in each experiment were calculated in terms of per cent of the average values of the first three collection periods (first hour). On such a basis, the average per cent values of the volume of secretion and the output of free acid for each collection period (20 min.) have been represented graphically in figure 1. The maximum inhibition took place during the second 20-minute period following the injection of urogastrone in both the control and enterectomized dogs. During the period of maximum inhibition, the volume of secretion was reduced by 43.9

per cent in the controls and 68.6 per cent in the enterectomized animals; the output of free acid was reduced by 50 per cent in the controls and 80.6 per cent in the enterectomized animals. These data indicate the similarity between the response of the enterectomized and control dogs to urogastrone. If any difference did exist, the degree of inhibition was greater in the enterectomized dogs.

DISCUSSION. The evidence obtained in these experiments indicates that the inhibitory effect of urogastrone on gastric secretion is not dependent upon the
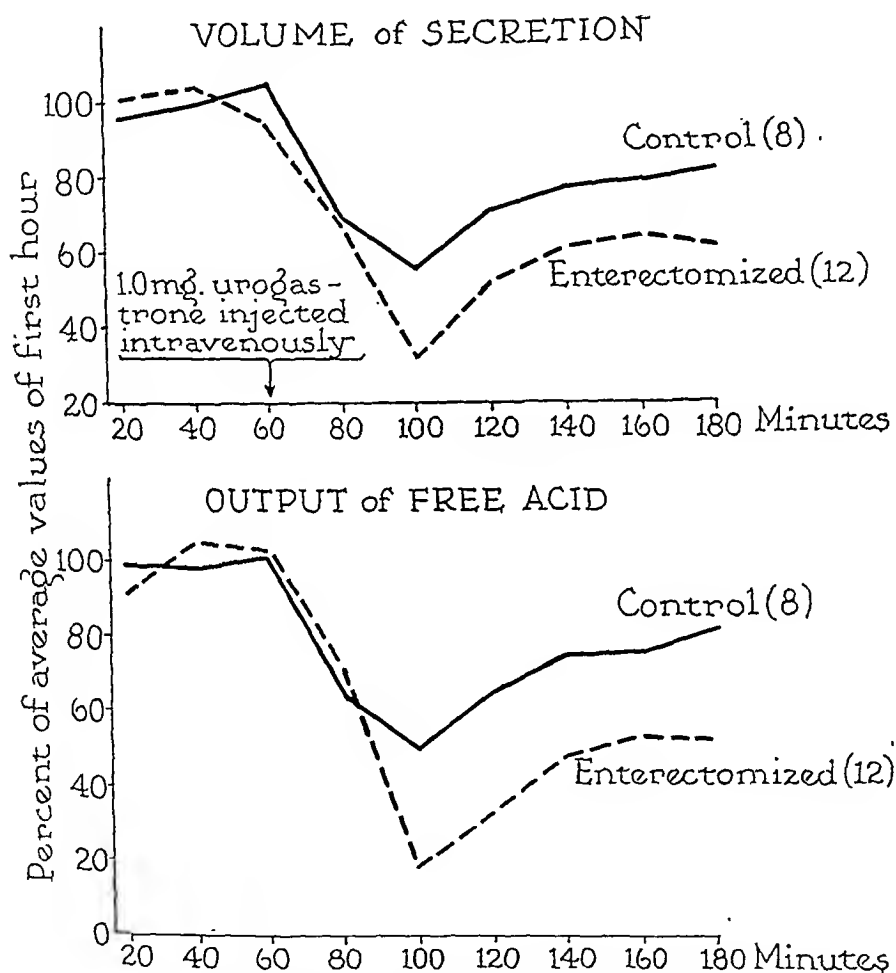
Fig. 1. 0.15 mgm. histamine dihydrochloride was injected subcutaneously at 10-minute intervals throughout the experimental period.

small intestine. Therefore, it is highly improbable that urogastrone acts by liberating enterogastrone, one of the hormones of the small intestine. The reasons for the disagreement between these results and those of Friedman (1941) are probably to be found in the differences in experimental procedure. Friedman employed acutely enterectomized animals under sodium pento-barbital anesthesia and used variable doses of urine extracts prepared in a different manner from those employed in this study.

CONCLUSIONS

1. The intravenous administration of 1.0 mgm. of urogastrone effectively inhibited the volume of gastric secretion and the output of free acid in 12 experiments on 6 enterectomized dogs and in 8 experiments on 8 dogs with vagotomized total gastric pouches.

2. No evidence was obtained which would indicate that urogastrone inhibits gastric secretion through the liberation of enterogastrone.

REFERENCES

FRIEDMAN, M. H. F.   This Journal **133**: P283, 1941.
GRAY, J. S., E. WIECZOROWSKI, J. A. WELLS AND S. C. HARRIS.   Endocrinology **30**: 129, 1942.

THE PENETRATION OF SUGARS INTO THE AQUEOUS HUMOUR

C. B. WELD, W. H. FEINDEL[1] AND H. DAVSON

From the Department of Physiology, Dalhousie University, Halifax, N. S.

Recent work (10, 4) has shown that the polysaccharide inulin does not penetrate the blood-aqueous barrier under normal conditions although it may do so when the permeability of the membrane is increased by paracentesis (4) or by instilling certain vasodilatory drugs into the eye (9). The present investigation was carried out with the purpose of defining the limiting molecular size of lipoid insoluble non-electrolytes penetrating the membranes which separate the aqueous humour of the eye from the blood. A comparison was made of the penetrating properties of four sugars with different molecular sizes, namely, xylose, glucose, sucrose and raffinose.

METHODS. Dogs were anesthetised with sodium amytal and injected intravenously with a solution of the sugar being studied. A specimen of aqueous humour was obtained from one eye before injection and from the other eye at a desired interval after injection. In a few instances where a considerable increase in aqueous sugar was expected no aqueous was taken before injection of the sugar, the two specimens being taken at suitable intervals later. In our experiments the reducing value of the aqueous was consistently close to that of the venous serum, averaging 3.5 per cent lower. In these experiments the initial aqueous value was taken as 3.5 per cent less than the initial serum value. It was felt that the error involved in this assumption was not great as the increase in the aqueous reducing value was 50 per cent or more and that it was outweighed by the advantages of obtaining two points on the curve with the one animal. Specimens of venous blood were taken before injection and at various periods during the course of the experiment; after clotting, these were centrifuged. Determinations of reducing values were made on 0.2 cc. samples of aqueous humour and of serum by the method of Hagedorn and Jensen (7). The reducing values of sucrose and raffinose were taken as the increase after hydrolysis by N/10 sulfuric acid. Duplicates agreed to within 3 per cent.

For short periods, up to twenty minutes, a single injection of an isotonic solution of the sugar was given in sufficient quantity to raise the blood sugar level to two or three times the normal. Over longer periods it was necessary to use a slow continuous injection of a more concentrated solution to maintain the increased blood level. It will be noted that in the case of the sucrose figures, the rise in serum levels is somewhat greater, in spite of a much slower injection rate. A comparison of the two results for the 30 minute interval would indicate that this factor is unimportant. Of thirty completed experiments the results of six were rejected because the blood sugar levels either were not raised sufficiently or were not satisfactorily maintained during the period of injection.

RESULTS. The reducing values were calculated in terms of milligrams per cent of glucose. The ratio R, where R equals

$$100 \times \frac{A_1 - A_0}{S_1 - S_0}$$

is to be plotted against the time after injection at which the second aqueous sample was taken. A_0 and S_0 are the pre-injection reducing values of aqueous and serum respectively. A_1 is the reducing value of the second aqueous sample and S_1 is the average reducing value of the serum during the period of injection. The ratio R gives us a measure of the concentration gradient between the serum and aqueous humor.

Table 1 shows the values obtained in two experiments with glucose. These are selected as being typical of results obtained. The second example is one

TABLE 1

Glucose

TIME AFTER 1ST INJECTION	FLUID	REDUCING VALUE	INCREASE IN SUGAR	AVERAGE SERUM INCREASE	R
minutes					
0	Aqueous	74			
90	Aqueous	186	112		
0	Serum	76			
3	Serum	210	134		
30	Serum	267	191		
60	Serum	222	146		
90	Serum	270	194	166	65
0	Aqueous	98			
65	Aqueous	174	76		
0	Serum	93			
3	Serum	413	271		
27	Serum	282	140		
65	Serum	155	13		

discarded because the serum reducing value was not maintained during the experiment. The first is given as a straightforward result: there is nothing in the experiment to indicate an error. Table 2 summarizes the results of all four sugars.

If the results R are plotted against time it becomes apparent that both the initial time of penetration and the subsequent increase in concentration of the four sugars in the aqueous humour is inversely related to the molecular weight. Thus for xylose with a molecular weight of 150, a rapid rate of penetration was found and the decrease in concentration gradient between the blood and the eye was about 25 per cent at three minutes after injection.

Glucose, with a molecular weight of 180, behaved in much the same manner with a decrease in concentration gradient of about 25 per cent in ten minutes. Sucrose diffusion was much slower, the concentration gradient being decreased

by about 25 per cent only after three hours. The penetration of raffinose in one hour is almost negligible, i.e., it is within the error of titration. At three hours the penetration is measurable but so low as to indicate that raffinose, with a molecular weight of 504, approaches the limiting size of a lipoid insoluble non-electrolyte molecule penetrating the blood aqueous barrier.

DISCUSSION. Since the difference between the successive members of the series glucose, sucrose and raffinose is represented by one hexose unit it is justi-

TABLE 2

TIME	SUGAR	INCREASE IN AVERAGE SERUM AFTER INJECTION	INCREASE IN AQUEOUS	VALUE FOR R
1.5 min.	Xylose	163 mgm. %	40 mgm. %	25
5 min.	Xylose	165	39	24
15 min.	Xylose	150	52	35
30 min.	Xylose	142	65	46
60 min.	Xylose	113	68	60
60 min.	Xylose	175	85	49
60 min.	Xylose	103	46	43
90 min.	Xylose	1164	112	68
8 min.	Glucose	196 mgm. %	39 mgm. %	20
14 min.	Glucose	177	58	37
16 min.	Glucose	234	73	31
16 min.	Glucose	212	71	33
30 min.	Glucose	187	85	45
1 hour	Glucose	236	103	44
90 min.	Glucose	166	112	65
120 min.	Glucose	202	60	30
200 min.	Glucose	237	92	39
15 min.	Sucrose	373	0	0
19 min.	Sucrose	402	8	2
30 min.	Sucrose	278	15	5
30 min.	Sucrose	651	44	7
2 hours	Sucrose	343	34	10
3 hours	Sucrose	432	105	24
1 hour	Raffinose	195 mgm. %	4 mgm. %	2
3 hours	Raffinose	245	10	4

fiable to assume that the molecular weight of these sugars is indicative of molecular size. In the case of the polysaccharide inulin one axis of the molecule is believed to be some ten times the length of the other axis. Hence the molecule has a low diffusion co-efficient and behaves as though it has a molecular weight of the order of 15,000 (1) instead of 5000 (11) which other methods give it. It is reasonable to assume that the disaccharides and trisaccharides have a molecule much more spherical and more proportional in size to their molecular weight than has inulin.

That molecular size is a determining factor in penetration through certain organized cellular membranes has been demonstrated by Chambers (2, 5). Admittedly less importance is attached to this factor in the case of leaky membranes like the capillary endothelium, which allows substances to pass through large intercellular pores, than for closely packed cell layers such as are found in the proximal tubules of the kidney. In the vascular structures which are the sites of the aqueous formation in the eye, the blood is separated from the aqueous humour by capillary endothelium, the membrane of Bruch, and pigmented epithelium. Here it would be expected that the factor of molecular size would largely determine the penetration of a substance from the blood into the aqueous.

It seems probable that xylose and glucose are capable of passing from the blood to the aqueous humour both through the cells as well as through intercellular pores while the slow penetration of sucrose and raffinose is explained by their passage only through intercellular pores. In the case of raffinose, the actual size of the molecule must approximate sufficiently closely to the pore size as to be a limiting factor in the penetration of this sugar.

It has been shown that the osmotic pressure of the aqueous humour is measurably greater than that of the blood plasma (3, 6). The concentration of chloride in the aqueous humour is also greater than that expected on the basis of calculation and is not due to metabolic activity of the lens or to the variations in the blood chloride level (4).

These discrepancies from the distribution ratio expected upon the basis of a filtration process are not found in the fluid formed after paracentesis (4) and are abolished by poisoning the eye tissues (8), facts which suggest that in the formation of the aqueous humour, some secretory activity is imposed upon the primary process of filtration. For this to be true the structure of the blood-aqueous humour barrier must be such that its intercellular spaces are small enough to prevent the ready escape of the secreted substances back into the blood; otherwise the establishment of a concentration gradient would be virtually impossible. That this structural basis for the validation of a secretory process is provided for is shown by the relative impermeability of the eye membranes to the comparatively small molecule of raffinose.

It will be noted that the glucose curve is more irregular than the others and except for one single result (the 90 min. period; see also table 1) there is a suggestion that though glucose enters the eye rapidly there is some mechanism preventing excessive accumulation in the aqueous humour. This may indicate some property of the eye but on the other hand it may merely be a reflection of the greater difficulty encountered with this sugar in maintaining the high serum level. Despite the injection of some 100 cc. of 18 per cent glucose per hour the serum levels of four of these dogs failed to be maintained. It is felt that because of this there may have been changes in pressure relationships sufficiently great to affect the permeability of the blood-aqueous barrier. In the absence of intra-ocular and of blood pressure determinations in these animals such a suggestion is mere surmise but nevertheless there is some room for doubt about the interpretation of the latter part of the glucose curve.

CONCLUSIONS

1. Penetration of the sugars xylose, glucose, sucrose and raffinose from the blood stream into the aqueous humour is inversely related to their molecular size.

2. Raffinose, with a molecular weight of 504, represents the limit of the size of lipoid insoluble nonelectrolyte molecule entering the eye.

3. The relative impermeability of the eye membranes to raffinose is compatible with the theory of a secretory activity being imposed upon the primary process of filtration in the formation of the aqueous humour.

REFERENCES

(1) BUNIM, J. J., W. W. SMITH AND H. W. SMITH.   J. Biol. Chem. **118**: 667, 1937.
(2) CHAMBERS, R.   Symposia on Quantitative Biology **8**: 144, 1940.
(3) DAVSON, H., W. S. DUKE-ELDER AND G. H. BENHAM.   Biochem. J. **30**: 773, 1936.
(4) DAVSON, H. AND C. B. WELD.   This Journal **134**: 1, 1941.
(5) GORDON, H. K. AND R. CHAMBERS.   J. Cell. Comp. Physiol. **17**: 97, 1941.
(6) HODGSON, T. H.   J. Physiol. **94**: 118, 1938.
(7) PETERS, J. P. AND D. D. VAN SLYKE.   Quantitative clinical chemistry.   Vol. 2, 471.
     Williams and Wilkins, Baltimore, 1932.
(8) ROEPKE, R. R. AND W. A. HETHERINGTON.   This Journal **130**: 340, 1940.
(9) SWAN, K. C. AND W. M. HART.   Am. J. Ophthalmol. **23**: 1311, 1940.
(10) WELD, C. B., H. DAVSON AND W. H. FEINDEL.   This Journal **133**: 485, 1941.
(11) WESTFALL, B. B. AND E. M. LANDIS.   J. Biol. Chem. **116**: 727, 1936.

THE EFFECT OF PEPTONE ON CAPILLARY PERMEABILITY AND ITS NEUTRALIZATION BY ADRENAL CORTICAL EXTRACT[1]

I. H. SHLESER AND S. C. FREED

From the Cardiovascular and Medical Departments, Michael Reese Hospital, Chicago

The intravenous injection of many foreign substances and of tissue extracts as well as peptone has long been known to lead to a condition of peripheral vascular collapse (1, 2, 3, 4) associated with capillary dilatation, increased capillary permeability, and leakage of plasma (2, 5) leading to a decreased circulating blood volume and hypotension. In this state, the essential factor involved appears to be capillary-wall damage leading to the increased permeability, and this has been attributed to the secondary liberation of histamine (5).

It has been postulated by Swingle and his associates (6, 7) that the action of adrenal cortical extracts in surgical and other types of shock is in large part to prevent such an increase in capillary permeability. Experimental evidence supporting this view has been advanced by the work of Menkin (8) who has shown that in the rabbit adrenal cortical extract prevents the increase in permeability of the skin capillaries caused by leukotaxine. This observation has been confirmed by us (9). We found that this neutralizing action was not obtained with desoxycorticosterone but did occur with corticosterone.

It appeared to us that it would be desirable to repeat the observation on capillary permeability with the method of Menkin (8), using peptone as a test substance instead of leukotaxine. Such observations on the capillaries of the rabbit, as measured by dye accumulation in a local area of the skin, would serve to test directly *a*, the action of peptone, and *b*, the possible neutralizing effect of adrenal extract and some of the adrenal steroids.

METHODS. In twenty-three rabbits an intracutaneous injection of 0.2 cc. of a 2 per cent solution of Difco Bacto-peptone was followed ten minutes later by an intravenous injection of 10 cc. of $1\frac{1}{2}$ per cent solution of trypan blue. A comparison of the color of the skin over the site of injection with that of the surrounding area gave an indication of the amount of dye accumulating in the tissues at this point. A 4 plus reaction was considered one that gave an intense blue coloration over the site of injection. Less intense color concentrations were graded down to 1 plus; a doubtful accumulation of dye was noted as ? plus; no accumulation as 0, and blanching of the area of injection by −1. Because of the variability in response of rabbits, the effects were compared on the same rabbit. For this purpose, the various adrenal cortical preparations were tested at the same time in different regions of the denuded skin, both when used alone or in combination with peptone. In the latter case the same amount of peptone was used as in the test with peptone alone. When the adrenal cortex extract was used

0.1 cc. of the commercial preparation (Wilson)[2] was injected. In the case of the other preparations of adrenal cortex tested, i.e., corticosterone, compound E, and anhydrohydroxyprogesterone[3] which are relatively insoluble in water, solutions were made as follows: The steroids were dissolved in a small quantity of alcohol, physiological saline was added and the alcohol driven off by heating in a water bath. The material remained in solution when the temperature was near the boiling point, but at room temperature much, but not all, of the material recrystallized to form a suspension. Since the amount recrystallized was less than the original, the remainder was in solution or in a very fine suspension. After cooling, the final mixture was diluted until each cubic centimeter of solution con-

TABLE 1

The effect of peptone and of corticosterone, compound E and adrenal cortical extract, with and without peptone, on the local accumulation of trypan blue in the rabbit's skin

CORTICOSTERONE	CORTICOSTERONE WITH PEPTONE	COMPOUND E	COMPOUND E WITH PEPTONE	PEPTONE	ADRENAL CORTICAL EXTRACT WITH PEPTONE
0	+++	0	++++	+++	−1
0	++	0	++	++	−1
0	0	0	++	++	−1
+	+++	+++	++++	++++	+
+++	+++	++	++	++	0
?+	++	0	++	+++	0
0	0	0	0	++	0
		+++	+++	++	−1
		0	+	+	0
		+	+++	++	+
		+	++	++	
		+	++	++	0
		0	+	++	0
			+++	+	0
	++			0	
	++			0	
	++			++	
	++			++	
				++++	0

Each line across represents observations on one rabbit.

tained from 2 to 5 mgm. of the steroid. Desoxycorticosterone acetate was not used because of its lack of action on the leukotaxine effect (9).

RESULTS. The results are summarized in tables 1 and 2. It will be noted that the peptone solution caused an increased skin capillary permeability in 20 of the 23 animals tested, the response ranging in the positive results from 1 plus to 4 plus. It is this variability in results that makes it essential in noting the

effects of the corticosteroids to compare them with the control on the same animal. This was the procedure followed in this study. The results in table 1 show that adrenal cortical extract prevented the manifestation of increased capillary permeability of the peptone when it was injected simultaneously with it. The occasional blanching noted occurs also when adrenal cortical extract is injected alone (9).

None of the adrenal cortex steroids nor anhydrohydroxyprogesterone duplicated the action of the cortical extract since they failed in most instances to neutralize the peptone effect, as tables 1 and 2 show.

The results with peptone differ from those reported with leukotaxine in that the neutralizing effect previously observed (9) when corticosterone was added to leukotaxine was not found when it was added to peptone. In order to check this discrepancy the experiments with leukotaxine[4] and corticosterone were repeated.

TABLE 2

The effect of anhydrohydroxyprogesterone, with and without peptone, on the local accumulation of trypan blue in the rabbit's skin

ANHYDROHYDROXY-PROGESTERONE	ANHYDROHYDROXY-PROGESTERONE WITH PEPTONE	PEPTONE
++	+	+
?+	+++	0
0	+++	+
++	++	++

Each line across represents observations on one rabbit.

TABLE 3

Effect of corticosterone on the leukotaxine action in causing accumulation of trypan blue in the rabbit's skin

CORTICOSTERONE WITH LEUKOTAXINE	LEUKOTAXINE
+++	++++
+++	++++
+++	+++
+++	+++
0	++
?+	++
0	++
?+	++

Each line across represents observations on one rabbit.

The neutralizing effect of corticosterone was manifest mainly in those animals in which the leukotaxine by itself gave the least effects (table 3). The neutralizing action of corticosterone against leukotaxine is apparently not powerful, and is noteworthy only by comparison with desoxycorticosterone acetate which is ineffectual even when used against weak solutions of leukotaxine.

DISCUSSION. The fact that corticosterone consistently *failed to prevent weak* reactions to peptone, while preventing weak reactions to leukotaxine, suggests that the capillary damaging factor in the peptone solution is not leukotaxine but some substance with a leukotaxine-like action. This supposition is not unlikely since we have repeatedly observed leukotaxine-like reactions following the injection of substances in no way related to protein catabolism. Thus, a preparation

[4] We are indebted to Dr. V. Menkin for generously supplying us with a quantity of fresh leukotaxine.

of commercial gum acacia (6 per cent) was found to produce an accumulation of dye in the rabbit's skin. Apparently therefore the leukotaxine effect on capillary permeability is shared by peptone and other protein breakdown products (10) and is not a specific but rather a generic response of capillaries to some noxious agents. If such an action on skin capillary permeability is typical of effects throughout the body, it may possibly be involved in some of the untoward reactions occasionally encountered with some gum acacia preparations used in the treatment of shock (11).

Our experiments demonstrate that the intracutaneous injection of peptone increases the permeability of capillaries and therefore lend support to the view that peptone has a like action in other regions. Our results do not reveal the magnitude of these changes, and thus do not indicate to what degree this increase in capillary permeability contributes to the development of peripheral vascular collapse induced by the intravenous injection of peptone.

On the other hand, our present results with adrenal cortex extract, confirming our previous results (9), suggest that this substance tends to decrease capillary permeability and prevents the increased capillary permeability by substances like peptone and leukotaxine. It is therefore possible that the adrenal cortical extract may have a beneficial action in those cases of shock in which it can be demonstrated that increase in capillary permeability plays a significant rôle.

This action of adrenal cortical extract is not manifest by the cortical steroids tested (other than the slight effect noted by corticosterone on leukotaxine). Since the amounts of material able to act in the case of these steroids is unknown the difference may be quantitative and not qualitative. Nevertheless our work strongly suggests that the adrenal cortical extract contains some principle other than the steroids tested which has the specific property of preventing the increase in capillary permeability.

The fact that a cortical steroid does not affect capillary permeability does not exclude its possible utility in states of shock since work from this laboratory (12) has shown that desoxycorticosterone acetate, having no apparent neutralizing action on capillary permeability (9), nevertheless has a beneficial prophylactic action on the shock-like state induced by venous occlusion of a limb in the dog.

SUMMARY

1. Peptone induces an increase in permeability of the capillaries of the rabbit skin similar to that of leukotaxine.

2. This capillary damaging factor of peptone, like that of leukotaxine, can be neutralized more or less completely by adrenal cortical extract. Corticosterone, compound E and anhydrohydroxyprogesterone, however, do not have this effect.

3. Certain differences in the reaction of corticosterone to peptone and to leukotaxine suggest that the action of peptone is not due to leukotaxine but to a leukotaxine-like substance.

ADDENDUM: Since the preparation of this paper, our attention has been directed to an article by Duthie and Chain (Brit. J. Exp. Path. **20**: 417, 1939) in which is reported that peptic digests of a number of proteins such as serum, albumin, fibrin and casein all give a leukotaxine reaction.

REFERENCES

(1) HEIDENHAIN, R.   Pflüger's Arch. **49**: 252, 1891.

(2) PHEMISTER, D. B. AND J. HANDY.   J. Physiol. **64**: 155, 1927.

(3) SIMONDS, J. P.   J. A. M. A. **69**: 883, 1917.

(4) MARKOWITZ, C. AND F. C. MANN.   This Journal **96**: 709, 1931.

(5) DRAGSTEDT, C. A., F. B. MEAD AND S. W. EYER.   J. Pharmacol. and Exper. Therap. **63**: 400, 1938.

(6) SWINGLE, W. W., W. M. PARKINS, A. R. TAYLOR AND H. W. HAYS.   This Journal **123**: 659, 1938.

(7) SWINGLE, W. W., H. W. HAYS, J. W. REMINGTON, W. D. COLLINS AND W. M. PARKINS. This Journal **132**: 249, 1941.

(8) MENKIN, V.   This Journal **129**: 291, 1940.

(9) FREED, S. C. AND E. LINDNER.   This Journal **134**: 258, 1941.

(10) ROCHA E SILVA, M. AND C. A. DRAGSTEDT.   J. Pharmacol. and Exper. Therap. **73**: 405, 1941.

(11) STUDDIFORD, W. E.   Surg., Gynec. and Obstet. **64**: 772, 1937.

(12) KATZ, L. N., S. T. KILLIAN, R. ASHER AND S. PERLOW.   This Journal (in press).

A COMPARISON OF THE BLOOD PICTURES OF ACTIVE AND HIBERNATING GROUND SQUIRRELS

JOSEPHINE STUCKEY AND R. M. COCO

From the Department of Zoology, Physiology and Entomology, Louisiana State University, Baton Rouge

Few investigators have reported on the changes which occur in the blood of mammals during hibernation. Endres (1) found that the oxygen saturation of the arterial blood of hibernating marmots ranged from 93.5 to 96.4 per cent; the blood sugar varied from 71.0 to 96.0 mgm. per 100 cc. of blood as compared to 97.0 to 162.0 mgm..in the active state. The latter figures, he felt, were too high since no anesthesia was used and the animals became extremely excited when the blood was being obtained for blood sugar determinations. Stormont, Foster and Pfeiffer (2) reported an increase of 40.2 volumes per cent in the carbon dioxide content of the arterial blood of hibernating ground squirrels, and a corresponding change in pH from 7.43 to 7.10.

It is well known that the flow of blood during hibernation is greatly altered and the question naturally arises as to what changes, if any, occur in the composition of the blood. In an attempt to answer this question, the authors have undertaken to compare the blood pictures of active and hibernating ground squirrels.

METHODS. The animals used in this study were *Citellus tridecemlineatus* (Mitchill) and were obtained from west-central Kansas and the region around Milford, Iowa. They had been kept in the laboratory for at least one year before being used for experimental purposes. The active animals were kept under standard laboratory conditions throughout the year. Hibernation was induced, during the winter months when it would occur normally in the field, by placing the animals in individual metal cages containing ample bedding material and then placing the cages in a cold room the temperature of which ranged from 40 to 42 degrees Fahrenheit. The blood used in the determinations was obtained, with few exceptions, by cardiac puncture, while the animals were still in the cold room. The active animals were given just enough nembutal to stupefy them sufficiently to allow the withdrawal of blood without struggling and undue excitement; no anesthesia was necessary in case of the hibernating ones.

Routine hematological techniques were employed in making the red cell, white cell and differential counts. Hemoglobin determinations were made by the acid-hematin method using the Hellige-Wedge hemometer. Hammerschlag's method was used to determine the specific gravity. For the determination of the creatinine and glucose contents, the colorimetric methods of Folin and Wu were employed. The Folin-Wu method of blood glucose determination was checked against the more accurate method of Somogyi, Schaffer and Hartman. , The former method, even though it gave higher values (about 20 mgm. per 100 cc. of blood), required less blood, and for that reason was finally adopted for the routine

431

work with the ground squirrel.   In this paper the term "blood picture" is used to include the total red and white cell counts, the differential count, the hemoglobin content, the specific gravity, and the glucose and creatinine contents.   In order

TABLE 1

The blood picture of active ground squirrels

ANIMAL NO.	SEX	AVER-AGE WEIGHT*	RBC	WBC	DIFFERENTIAL (PER CENT)					SPECIFIC GRAVITY	HB	CREATI-NINE	GLUCOSE
					Neutro.	Baso.	Acido.	Lym-pho.	Mono.				
		grams.	mil. per cu. mm.	thou. per cu. mm.							gm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
I-1	F	184.0	9.500	4.766	51.5	0.0	1.6	38.6	8.3	1.051	13.4	1.500	123.0
I-2	F	126.5	4.968	5.800	51.6	0.0	1.3	34.5	12.6	1.052	13.3	1.151	105.0
I-3	F	130.0	7.793	4.675	59.7	0.0	1.5	31.3	7.5	1.049	14.0	1.486	102.9
I-4	M	92.5	6.942	3.188	60.0	0.0	4.0	32.0	4.0	1.051	14.0	1.203	120.8
I-5	F	95.4	8.343	6.543	59.0	0.0	1.0	28.0	12.0	1.050	15.0	2.300	122.1
II-1	F	291.3	10.318	3.293	52.3	0.0	2.0	38.4	7.3	1.058	15.6	1.310	100.2
II-2	F	286.6	8.830	2.716	55.6	0.0	1.3	34.1	9.0	1.058	14.9	1.158	101.5
II-3	M	261.7	8.944	2.975	65.5	0.0	1.0	29.0	4.5	1.062	15.7	1.405	106.9
II-4	F	256.2	13.200	2.400	62.0	0.0	2.0	29.0	7.0	1.059	13.1	1.230	121.9
II-5	F	257.5	9.672	1.150	50.0	0.0	4.0	40.0	6.0	1.061	13.4	1.610	100.3
II-6	F	241.5	7.914	2.025	70.0	0.3	1.7	17.5	10.5	1.059	15.0	1.540	111.9
I-6	F	265.0	9.348	2.280	60.5	0.0	3.0	31.5	5.0	1.058	15.0	1.131	125.0
III-1	M	280.0	8.419	1.984	58.0	0.0	1.4	30.4	10.2	1.058	13.1	1.229	127.0
III-2	M	310.7	6.304	3.900	65.0	0.2	1.4	25.6	7.8	1.059	14.7	1.588	111.9
III-3	F	294.6	6.965	2.700	61.5	0.0	1.4	28.6	8.5	1.058	11.7	1.649	117.5
III-4	M	219.0	6.992	3.900	74.0	0.0	0.8	20.2	5.0	1.056	16.2	1.588	116.7
III-5	M	347.3	9.402	4.228	65.4	0.0	1.6	30.6	2.2	1.062	15.8	1.265	98.2
III-6	M	293.1	8.250	2.710	63.3	0.0	2.4	23.1	11.2	1.059	15.8	1.432	97.3
IV-1	M	431.3	8.346	1.670	55.8	0.0	0.4	33.8	9.0	1.062	13.2	1.345	92.3
IV-2	M	371.1	8.356	10.848	58.0	0.0	1.8	32.6	7.6	1.058	12.9	1.377	99.4
IV-3	M	347.3	6.373	3.950	51.0	0.0	1.0	35.0	13.0	1.061	12.4	1.337	102.9
IV-4	M	371.2	7.460	8.240	54.6	0.0	2.0	32.4	11.0	1.058	13.5	1.314	97.2
IV-5	M	331.3	8.312	4.164	57.4	0.0	1.8	30.2	10.6	1.055	13.1	1.223	100.2
IV-6	M	221.2	7.752	3.924	54.0	0.0	1.4	34.8	9.8	1.057	14.2	1.560	93.9
V-1	F	383.9	7.565	3.920	55.5	0.0	1.4	37.9	5.2	1.062	16.3	1.335	104.5
V-2	F	281.1	6.698	6.654	54.2	0.0	1.4	34.8	9.6	1.058	13.3	1.364	102.5
V-3	M	360.1	7.557	3.960	54.0	0.0	0.8	34.4	9.8	1.062	15.9	1.329	101.5
V-4	F	445.5	7.614	3.892	53.6	0.0	1.2	36.6	8.6	1.062	16.4	1.318	102.9
V-5	M	422.4	7.451	3.876	57.8	0.0	2.0	32.2	8.0	1.061	16.1	1.368	104.5
V-6	F	334.7	7.457	3.532	51.8	0.0	1.0	38.6	8.6	1.061	16.0	1.395	103.0
Average ...		284.5	8.102	3.995	58.1	0.0	1.7	31.8	8.5	1.058	14.4	1.401	107.1

* The weight given for each animal is the average of the weights recorded approximately a month apart from October 1, 1941 to April 1, 1942.

to establish a norm, four to nine determination of each of these were made upon each of 30 active or normal animals at various times between October and April. After sufficient time had elapsed to insure complete recovery from any effects of cardiac puncture, 18 of these animals were placed in the cold room for periods of

time varying from 8 to 28 days and induced to hibernate. After the periods indicated, determinations were again made. Thus it was possible to compare the average blood pictures of these 18 hibernating animals to that of 30 active ones and, since the blood picture of each hibernating animal was known before hi-

TABLE 2

The blood picture of hibernating ground squirrels

ANIMAL NO.	SEX	DAYS, C. ROOM	WEIGHT	RBC	WBC	DIFFERENTIAL (PER CENT)					SPECIFIC GRAVITY	HB	CREATININE	GLUCOSE
						Neutro	Baso.	Acido.	Lympho.	Mono.				
			grams *	*mil. per cu. mm.*	*thou. per cu. mm.*							*gm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
III-3	F	8	264.2	4.430	3.200	24.0	0.0	3.0	44.0	29.0	1.054	9.2	1.430	80.5
III-5	M	10	326.0	6.840	3.420	30.0	0.0	3.0	40.0	26.0	1.059	12.2	1.197	89.1
III-6	M	10	238.2	6.904	3.200	32.0	0.0	3.0	41.0	24.0	1.054	12.9	1.174	78.1
IV-1	M	12	362.1	6.430	3.550	58.0	0.0	3.0	21.0	18.0	1.058	12.4	1.115	81.3
IV-3	M	12	321.3	5.820	3.300	30.0	0.0	3.0	40.0	27.0	1.058	12.1	1.219	81.4
IV-4	M	12	336.0	5.376	7.750	34.0	0.0	4.0	42.0	20.0	1.057	12.2	1.241	82.3
IV-5	M	12	317.1	6.340	3.700	31.0	0.0	1.0	41.0	27.0	1.053	12.4	1.012	78.3
IV-6	M	12	205.1	6.840	3.200	39.0	0.0	1.0	41.0	19.0	1.056	13.1	1.318	70.9
II-1	F	23	262.0	4.348	2.500	30.0	0.0	2.0	42.0	26.0	1.046	9.1	1.000	87.1
II-2	F	23	175.5	4.346	3.800	38.0	0.0	1.0	41.0	20.0	1.046	9.0	1.000	76.2
II-3	M	23	135.5	3.480	3.900	58.0	0.0	3.0	30.0	10.0	1.043	8.5	1.104	81.2
II-4	F	23	166.5	6.450	3.100	42.0	0.0	1.0	44.0	13.0	1.056	11.5	1.168	81.3
II-5	F	23	103.5	6.556	2.300	32.0	0.0	3.0	48.0	16.0	1.057	11.5	1.120	71.2
II-6	F	23	216.0	5.640	2.250	60.0	0.0	4.0	20.0	16.0	1.054	10.5	1.213	78.9
I-1	F	28	124.2	3.200	5.650	25.0	0.0	3.0	43.0	29.0	1.039	9.1	1.300	72.4
I-2	F	28	104.5	3.600	5.430	52.0	0.0	2.0	35.0	11.0	1.038	9.1	1.121	90.3
I-3	F	28	99.0	2.300	6.200	32.0	0.0	3.0	40.0	26.0	1.036	8.2	1.248	70.9
I-6	F	28	186.0	1.720	3.800	39.0	0.0	2.0	42.0	17.0	1.028	9.0	1.120	106.6
Average (18 hibernating animals).....			218.9	5.034	3.900	38.1	0.0	2.5	38.6	20.8	1.050	10.7	1.172	81.5
Average (30 active animals)..			284.5	8.102	3.995	58.1	0.0	1.7	31.8	8.5	1.058	14.4	1.401	107.1
Per cent change (+ or −)			−23.0	−37.8	−2.3	−34.4	0.0	+47.0	+21.3	+144.7	−0.75	−25.0	−16.3	−14.5

* Weights taken after being in cold room for periods indicated when determinations were made.

bernation, each animal, as a double check, served as its own control (compare the same animal in tables 1 and 2).

RESULTS. Tables 1 and 2 give all pertinent data relative to the blood pictures of thirty active and eighteen hibernating ground squirrels, respectively. A study

of these tables will reveal the following changes in the blood pictures due to hibernation:

1. An average decrease of 37.8 per cent in the number of erythrocytes, and 25.7 per cent in the hemoglobin content.

2. An average decrease of 2.3 per cent in the total white cell count with the following changes in the differential count: a 34.4 per cent decrease in the number of neutrophils, no appreciable change in the percentage of basophils, an increase of 47.0 per cent in the acidophils which is probably not significant because of the smaller number of cells encountered, a 21.3 per cent increase in the lymphocytes, and an increase of 144.7 per cent in the monocytes. It should be stated at this point that in seven of the hibernating animals the total white cell count decreased and in eleven it increased. Probably more pronounced changes would occur if the animals were made to hibernate for longer periods.

3. A small but consistent decrease in the specific gravity of the blood amounting to 0.75 per cent.

4. A decrease of 16.3 per cent in the creatinine content.

5. A decrease of 14.5 per cent in the glucose content.

DISCUSSION. It is noted that the glucose content of the blood, as determined in this study, was lowered in every hibernating animal but that this decrease was in no case comparable to severe insulin hypoglycemia in which case the glucose values often range from 35 to 45 mgm. per 100 cc. of blood. For this reason it would seem that severe hypoglycemia is not the sole factor involved in inducing hibernation.

It is interesting to note that both the number of erythrocytes and neutrophils were depressed during hibernation, suggesting that there might be an inactivation of the red bone marrow. It is also possible, however, that some decrease in the number of erythrocytes was due to the storage of these cells in the spleen since it was reported by Evans that during hibernation in the bat the spleen acts as a reservoir for them. At the same time there was a pronounced increase in the monocytes and lymphocytes which would suggest that there might be an increased activity of the lymphoid tissue, or that the blood volume decreased and certain white cells remained in the circulating blood in full numbers. An investigation of the activities of the red bone marrow, the spleen and the lymphoid tissue during hibernation will be undertaken in subsequent studies.

The slight but consistent decrease in the creatinine values might have been due to the reduced metabolism at low temperatures, but nothing definite concerning this can be said at present, since the values were still within the normal range of one to two milligrams per 100 cc. of blood. It is probable that, for the periods indicated, the animals had not yet begun to utilize the proteins of the tissues.

SUMMARY

Induced hibernation in the ground squirrel, *Citellus tridecemlineatus* (Mitchill), for periods of eight to twenty-eight days, resulted in significant changes in the blood picture. These changes are: a decrease in the total red cell count and the hemoglobin content; a slight decrease in the total white cell count, a decrease in

the number of neutrophils, and an increase in the number of lymphocytes and monocytes; a slight decrease in the specific gravity; and a decrease in the creatinine and glucose contents.

REFERENCES

(1) ENDRES, G.   Proc. Roy. Soc. Series B **107**: 241, 1927.
(2) STORMONT, R. T., M. A. FOSTER AND C. PFEIFFER.   Proc. Soc. Exper. Biol. and Med. **42**: 56, 1939.

CEREBRAL METABOLISM IN FAT FED DOGS

ARTHUR G. MULDER AND LATHAN A. CRANDALL, JR.

From the Department of Physiology, University of Tennessee College of Medicine, Memphis

The results of experiments by a number of investigators (for review see Quastel (1)) indicate that the brain uses carbohydrate as its sole source of energy. This work has been relatively incomplete in regard to the ability of the brain to metabolize the ketone bodies. Goldfarb and Himwich (2) determined the arterio-venous differences in acetone body concentration in phlorizinized and depancreatized dogs. They found differences of less than 2.4 mgm. in most of their experiments. This work has not been confirmed.

Since these experiments were done there has been considerable improvement in the sensitivity of the methods by which the blood ketones can be determined. Furthermore, no experiments have been carried out on animals allowed to become habituated to the ketotic state; this might well be a factor determining the ability of the brain to use these substances. In view of this and because no experiments have been reported on fasting dogs it was thought that a redetermination should be undertaken.

The evidence that carbohydrate is the sole source of brain energy has been deduced partly from the determination of the respiratory quotient. In vivo experiments of Himwich and Nahum (3) gave an R.Q. of 1.0, those of Lennox (4) an R.Q. of 0.95 and Wortis, Bowman and Goldfarb (5) in a large series report an average R.Q. of 0.98. In vitro experiments have given the same results. It should be noted however that an R.Q. of approximately unity, while indicating no oxidation of fat, would be entirely consistent with the oxidation of ketone bodies since the theoretical R.Q. of aceto-acetic is 1.0 and of B-hydroxybutyric acid is 0.9. In view of the ready oxidation of the ketone bodies by other tissues (for literature see Crandall, Ivy and Ehni (6)) evidence obtained from the determination of the respiratory quotient cannot be assumed to be final.

As a result of in vitro experiments on this question Krebs and Johnson (7) and McGowan and Peters (8) have found that aceto-acetate produces an increase in oxygen uptake by brain tissue; the former authors suggested that ketone bodies are an intermediate in carbohydrate as well as in fat metabolism.

METHODS. The ketotic state was produced in dogs by starvation and fat feeding. After an initial two or three days of starvation the dogs were fed 100 cc. of olive oil daily. The period of starvation and fat feeding lasted from two to six weeks.

Arterial blood was drawn from the femoral artery. Venous blood was drawn from the superior longitudinal sinus, through a trephined opening in the skull. Eleven dogs were used. Nine of the animals were anesthetized with nembutal. In two dogs the trephined opening was prepared, under nembutal, 24 hours before the experiment, and the blood samples were drawn using local anesthesia (novo-

caine) only. These animals are listed as unanesthetized. In three experiments the blood was oxalated, in two animals calcamine fast pink was injected intravenously to prevent coagulation and in six of the animals heparin was used.

The blood was drawn directly into tonometers, displacing mercury, without contact with air. Arterial and venous samples were drawn at the same time and as nearly as was possible at the same rate. Blood for acetone body and glucose determinations was removed from the tonometer and the proteins precipitated immediately. The blood remaining in the tonometer, used for gas analysis, was kept in ice water. Ketone bodies were determined by the method of Crandall (9), glucose by the method of Schaffer and Somogyi (10), using the copper re-

TABLE 1

Cerebral metabolism in fat fed dogs

EXP. NO.	ANESTH. USED	ANTICOAG.	WEEKS FAT FED	KETONE BODIES AS B-HYDROXY (MGM./100 ML.)		A-V O₂ DIFF.	R.Q.	GLUCOSE (MGM./100 ML.)	
				Art.	A-V diff.			Art.	A-V diff.
						vol. per cent			
1	Nemb.	K. Ox.	2	6.4	−0.5				
				6.1	−0.5				
2	Nemb.	K. Ox.	2½	13.8	+0.1			120	−2
				11.4	−0.1			121	−2
3	Nemb.	K. Ox.	3	37.8	+0.7			51	−6
				30.3	+0.3			61	−8
4	Nemb.	C.F.P.	3½	35.8	+0.6	7.53	1.01	64	−7
5	Nemb.	C.F.P.	3	64.5	0	7.58	1.07	60	−15
6	Nemb.	Hep.	4	4.8	−0.7	5.9	0.96		
7	Nemb.	Hep.	4	4.5	+0.1	9.02	0.97	79	−16
8	Nemb.	Hep.	5	4.6	+0.2	13.42	0.95		
9	Nemb.	Hep.	6	64.3	−0.4	5.6	1.19		
10	None	Hep.	5	21.9	+0.4	11.25	0.96	62	−16
11	None	Hep.	2	13.9	+0.6	8.1	0.97		
Mean.........................					+0.057	8.55	1.01		−9.0
Standard error of mean.................					±0.12		±0.029		

agent for minimal sugar values. O_2 and CO_2 were determined by the method of Van Slyke and Neil (11).

RESULTS. Table 1 lists the experimental data. No arterio-venous difference greater than 0.7 mgm. per 100 ml. was found in the concentration of the acetone bodies. As the method has an accuracy of ±5 per cent all differences are within the range of the error of the experimental method. The mean arterio-venous difference was ±0.057. The standard error of the mean was ±0.12. The level of ketosis varied widely giving an opportunity to test ketone body utilization at various concentrations. The level of ketosis usually bore some relation to the duration of fat feeding.

The arterio-venous oxygen differences show variations from 6 vol. per cent to

13 vol. per cent, the variation probably depending at least in part upon the rate of blood flow through the brain. The respiratory quotients fell between 0.95 and 1.19. The mean is 1.01 with a standard error of ±0.029. If the exceptional value 1.19 found in experiment 9 is omitted the mean becomes 0.98 with a standard error of ±0.016.

DISCUSSION. Our results show definitely that there is no significant utilization, or production, of acetone bodies by brain under the conditions of our experiments. The results of Goldfarb and Himwich (2) are extended by our experiments to include animals which are well habituated to the ketotic state. It appears unlikely that there are any conditions in which the brain removes or adds acetone bodies. In a recent paper, Crandall, Ivy and Ehni (6) have suggested that ketosis may be regarded as a manifestation of a special type of metabolism occurring in the glucoprivic state, since acetone bodies are added to the blood by the liver only when the supply of glucose is inadequate and since the acetone bodies can replace glucose in some phases of metabolism. It is evident that acetone body production by the liver, which supplies a fuel which can be substituted for glucose by muscle tissue, does not serve the same purpose for the tissue of the central nervous system. In fact, the substitution of acetone bodies for glucose in muscle metabolism apparently serves to conserve glucose for the needs of the brain, which appears to be completely dependent upon this fuel.

Krebs and Johnson (7), as a result of in vitro experiments, have postulated that the ketone bodies are intermediates not only in fat metabolism but also in that of carbohydrate. They report an increase in oxygen uptake of sliced pigeon brain cortex in the presence of aceto-acetate. Our experiments clearly show that the brain in vivo does not use ketone bodies from the blood. Since these substances are readily diffusible it seems improbable that the brain itself produces acetone bodies and burns them as rapidly as they are produced. The fact that the respiratory quotient of the brain is unity indicates that they are not formed from fat for oxidation in situ. The respiratory quotients also indicate that there can be little or no direct burning of fat even after prolonged fat feeding.

The figures on the glucose used by the brain give a mean arterio-venous difference of 9 mgm. per 100 ml. This is practically identical with a value of 9.7 mgm. which we have obtained (unpublished data) in dogs in the postabsorptive state. Himwich and Nahum (3) have reported that the brains of depancreatized and phlorizinized dogs use glucose. Various observers have found mean arterio-venous differences of 9 mgm. (4), 11.2 (12), 13.5 mgm. (13), 12.5 mgm. (14) and 9 mgm. (5) of glucose in normal dogs and men. It is evident that the values which we have found vary little from normal values, indicating that the utilization of glucose by the brain is not markedly altered by the period of fat feeding.

The arterio-venous oxygen differences are also approximately the same as those reported by others. Various observers have found mean oxygen differences of 6.2 (4), 10.12 (3), 6.4 (13), 7.04 (14) and 6.9 (5) vol. per cent in dog and man. These results are reasonably in accord with the mean value of 8.55 vol. per cent found by us.

In our experiments as well as in the others cited above, the relation between the glucose difference and the O_2 difference is not exact. If we compare the four experiments in which blood sugar and oxygen differences were determined on the same samples we obtain a mean glucose utilization of 13.5 mgm. per 100 ml. which would require 10 vol. per cent of O_2. The actual mean O_2 difference in these experiments is 8.84 vol. per cent. If there is a lag between glucose removal and the oxidation of that glucose, variations in the rate of glucose removal or of blood flow could account for the lack of agreement.

SUMMARY

The brain of the dog which has been fed fat for a sufficient time not only to develop ketosis but to become habituated to the ketotic state does not burn acetone bodies.

The brain of the fat fed dog has an R.Q. of approximately 1.0.

The amounts of oxygen and glucose removed by brain during the ketotic state are not significantly different, in terms of milligrams per 100 ml. of blood, from those removed by the brain tissue of normally fed animals.

REFERENCES

(1) QUASTEL, J. H. Physiol. Rev. **19**: 135, 1939.
(2) GOLDFARB, W. AND H. E. HIMWICH. J. Biol. Chem. **101**: 441, 1933.
(3) HIMWICH, H. E. AND L. H. NAHUM. This Journal **90**: 389, 1929; **101**: 446, 1932.
(4) LENNOX, W. G. Arch. Neurol. Psychiat. **26**: 719, 1931.
(5) WORTIS, J., K. M. BOWMAN AND W. GOLDFARB. Am. J. Psychiat. **97**: 552, 1940.
(6) CRANDALL, L. A., JR., H. B. IVY AND G. J. EHNI. This Journal **131**: 10, 1940.
(7) KREBS, H. A. AND W. A. JOHNSON. Biochem. J. **31**: 645, 1937.
(8) McGOWAN, G. K. AND R. A. PETERS. Biochem. J. **31**: 1637, 1937.
(9) CRANDALL, L. A., JR. J. Biol. Chem. **133**: 539, 1940.
(10) SHAFFER, P. A. AND M. SOMOGYI. J. Biol. Chem. **100**: 695, 1933.
(11) VAN SLYKE, D. D. AND J. M. NEIL. J. Biol. Chem. **61**: 523, 1924.
(12) DAMESHEK, W., A. MYERSON AND C. STEPHENSON. Arch. Neurol. Psychiat. **33**: 1, 1935.
(13) HIMWICH, H. E. AND J. F. FAZEKAS. Endocrinology **21**: 800, 1937.
(14) HIMWICH, H. E., K. M. BOWMAN, J. WORTIS AND J. F. FAZEKAS. J. Nervous and Ment. Dis. **89**: 273, 1939.

POTENTIAL CHANGES IN INJURED CARDIAC MUSCLE

MASSIMO CALABRESI[1] AND ARTHUR J. GEIGER

From the Department of Internal Medicine, Yale University, New Haven, Connecticut

It has long been believed that the potential of injured heart muscle is constant and is maintained during systole at the resting negative level of potential. Therefore, it has traditionally been accepted that the monophasic character of the potential changes recorded electrographically with direct bipolar leads, one of which is placed upon the area of injury, is due entirely or predominantly to potential changes at the electrode over the uninjured area.

These views have been contested by Wilson and his associates (1) who showed by means of unipolar lead electrograms that during ventricular systole the potential of injured cardiac muscle does not remain constant at the resting value, and they presented evidence that the monophasic curve of action potential recorded from an injured area of heart muscle is, in fact, derived from important potential changes occurring at the area of injury whose participation had hitherto been considered negligible or absent. These observations were subsequently confirmed by Eyster and his collaborators (2), who also demonstrated that during activity the injured region actually changed from a negative to a positive potential with reference to the potential of uninjured resting ventricular muscle.

That these reports did not indisputably reverse the traditional concepts was apparent, however, from a publication by Jochim, Katz and Mayne (3). Their observations, based on transient cardiac injury produced by pressure, were interpreted as supporting the earlier view that the monophasic response obtained by leading off from injured and uninjured spots represents chiefly potential changes in the latter. However, in a report of later experiments from Katz' laboratory (4) the findings were considered to be in agreement with those of Wilson and of Eyster, but Katz and Jochim (5) adhered to the interpretation that "the potential changes are caused by alterations in the potential of the 'indifferent' electrode due to the changes in the state of polarization of the uninjured syncytial cell of the heart."

Because a solution of this question has an important bearing both on the theoretical concept of the genesis of electrical currents established by injury to the heart, and in electrocardiographic interpretation of acute heart muscle injuries (coronary thrombosis) in man, more work in this direction seemed desirable.

We have attacked the problem from a unique approach; namely, by comparing electrographic records of potential changes in injured hearts with direct measurements of potential differences through the use of a microvoltmeter.

ELECTROGRAPHIC OBSERVATIONS. Potential time curves (electrograms) were

---

[1] Dazian Foundation Fellow.

recorded directly from the exposed heart in experiments on nine frogs. The tracings were obtained through nonpolarizable silver-silver chloride electrodes connected with a standard string galvanometer electrocardiograph (Cambridge Instrument Co.); a measurable resistance was introduced into the circuit with the aid of a potentiometer when required to keep the size of the resultant electrograms within the dimensions of the film of the recording camera. Both unipolar and bipolar electrograms were obtained; in the former the lead contacts were at the cardiac apex (the site of subsequently induced injury) and at a distant point which was usually an exposed thigh muscle, and in the latter they were at the cardiac apex and base. The lead connections were so made that in the bipolar lead electrogram an upward deflection on the finished record represented a negative electrical response at the apex, while in the unipolar lead record an upward deflection represented a negative electrical response at
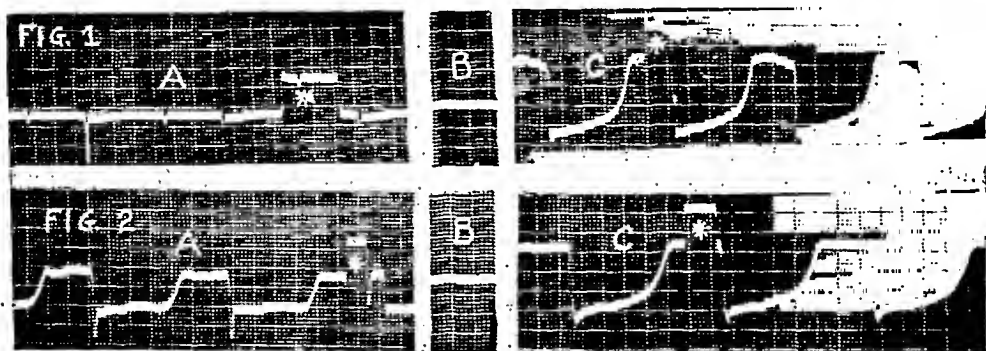
Fig. 1. Electrograms with unipolar lead (expt. 14 in table 1). A, control electrogram; B, zero potential baseline; C, electrogram after crushing injury under apical electrode. Asterisk indicates standardization of 3 mv.

Fig. 2. Electrograms with bipolar lead (expt. 15 in table 1). A, control electrogram; B, zero potential baseline; C, electrogram after cutting injury under apical electrode. Asterisk indicates standardization with 3 mv.

the exploring electrode. The potential of the uninjured resting ventricular muscle was taken as the reference level of potential.

Bipolar lead experiments. Figure 1 illustrates a typical result obtained consistently in seven experiments. It shows the essentially normal electrogram obtained before injury and the typical monophasic response recorded after production of a crushing injury at the cardiac apex. The upward displacement of the galvanometer string after injury reveals a resting injury potential of −16 mv. as compared with the resting potential in the uninjured state. The sharp downward deviation corresponding with the ventricular activity reveals that during systole the negative rest potential of the injured area is not only obliterated but that in addition a positive potential of +15 mv. is developed with reference to the potential of the uninjured resting ventricular tissue.

Unipolar lead experiments. Only two experiments were performed for the results were the same in each (fig. 2) and were consistent with the observations of others and with the electrograms we obtained with bipolar leads. When

the exploring electrode was placed in contact with the injured region the ventricular complex was monophasic in outline and was fundamentally similar to the electrograms obtained with bipolar leads. The change of potential in the positive direction was of sufficient magnitude to obliterate the negative injury resting potential and also to cause an additional positive deflection with respect to the reference potential.

The electrographic results of all nine experiments are summarized in table 1.

MICROVOLTMETER OBSERVATIONS. The availability of a vacuum tube microvoltmeter, recently devised by Burr, Lane and Nims (10), offered the opportunity of measuring direct current potentials in injured hearts; and information derived in this way was considered interesting as a means of verifying our electrographic observations of injury action potentials.

TABLE 1

Injury resting and injury action potentials in frog hearts

| FROG NO. | STANDARDIZATION | I. R. P.[†] MV. | I. A. P.[†] MV. | LEADS | | NATURE OF INJURY |
				Bipolar	Unipolar	
10*	8 mm = 1 mv.	−2(?)	+0.4(?)	+		Crushing
11	9 mm = 1 mv. K = 20	−8	+7	+		Cutting
12	5 mm = 1 mv. K = 20	−5	+8	+		Cutting
13	10 mm = 1 mv. K = 20	−24	+24	+		Cutting
14	9 mm = 3 mv. K = 20	−16	+15	+		Crushing
15	9 mm = 3 mv. K = 20	−16	+15		+	Cutting
16	1 cm = 3 mv. K = ?	−2	+6	+		Cutting
17	1 cm = 3 mv. K = 20	−13	+10	+		Cutting
18	1 cm = 1 mv. K = 100	−4	+4		+	Crushing
Average....................................		−10.0	+9.9			

* This single experiment was carried out with a Sanborn "Cardiette" electrocardiograph, and the standardization was not precisely controlled.

† I. R. P. refers to injury resting potential. I. A. P. refers to injury action potential.

The device employed is essentially a high sensitivity but relatively stable electrometer which, because of a high input impedance, draws virtually no current from the specimen under test. The designers state that the instrument is widely independent of resistance of the specimen, and that measurement of potential differences as small as 10 microvolts is made possible. Non-polarizable silver-silver chloride electrodes were used; each electrode was enclosed in a glass tube filled with 0.77 per cent saline solution and terminating in an opening of 1 to 2 mm. diameter that was plugged with a wick through which gentle contact with the heart was made.

The applicability of the microvoltmeter for observations of this nature was verified in experiments on exposed skeletal muscle in eleven frogs and two dogs. Direct current potentials were measured with a proximal electrode on the belly and a distal electrode near the tendinous insertion of the muscle. These measurements were repeated after a crosswise incision partway through the muscle

at the site of the proximal electrode. The results obtained appear in table 2; they show that a D.C. potential difference due to injury was readily detected by the instrument employed, and the values obtained were approximately of the expected order of magnitude (11).

Having established the fundamental validity of the microvoltmeter for the measurement of D.C. injury potentials in skeletal muscle, observations were undertaken on the exposed heart. Direct leads were obtained with one electrode in contact with the cardiac apex which was subsequently made the site of injury by cutting or crushing the tissue; the second electrode was placed either on the base of the heart (bipolar lead) or on an exposed but uninjured thigh muscle (unipolar lead). Unipolar lead records with the exploring electrode in

TABLE 2

D.C. potentials in skeletal muscle injured by cutting

ANIMAL	UNINJURED MUSCLE POTENTIAL	INJURED MUSCLE POTENTIAL	TOTAL DIFFERENCE
	mv.	*mv.*	*mv.*
Frog	+2	−12	−14
Frog	+1	−12	−13
	−2.5	−5.5	−3
	+2.5	−9	−11
	0	−5	−5
	−3	−16	−13
	+4	−13	−17
	+1	−14	−15
	−10	−24	−14
	−1	−10	−9
	−1	−12	−11
Average.............	−0.64	−12.0	−11.4
Dog	−1.5	−40.5	−39
Dog	−1.5	−45.0	−43.5

contact with the uninjured base of the heart and the indifferent electrode on the thigh muscle were also obtained for control.

Preliminary experiments on the exposed and beating hearts of the dog and cat revealed that the physical characteristics of the galvanometer in the microvoltmeter rendered it unsuitable for following the potential variations in such rapidly beating hearts. Our observations were therefore derived chiefly from the slowly beating exposed hearts of frogs.

In all such experiments on eleven frog hearts the following were consistently observed:

1. With both bipolar and unipolar leads from the surface of the uninjured and beating heart, the microvoltmeter showed either no deflections at all in systole, or at most a deflection of one millivolt with contraction. Since the validity of the technic had been established in the skeletal muscle experiments,

it appeared that the voltmeter galvanometer had too long a period adequately to record the changes of potential of the order of magnitude and duration encountered in the uninjured heart during systole.

2. Similar negligible changes in potential were observed after crushing or cutting the cardiac apex, provided the electrode was not within the area of injury. Apparently the presence of a fresh injury did not modify the potentials obtained from distant uninjured tissue.

3. When, on the other hand, the exploring electrode was actually touched to the injured site, a steady negative potential of several millivolts was recorded. This was to be anticipated from established knowledge that a fresh injury sets up a negative potential between injured and uninjured muscle.

Moreover, coincident with contraction of the heart this negative potential momentarily disappeared or declined from one to several millivolts. This might
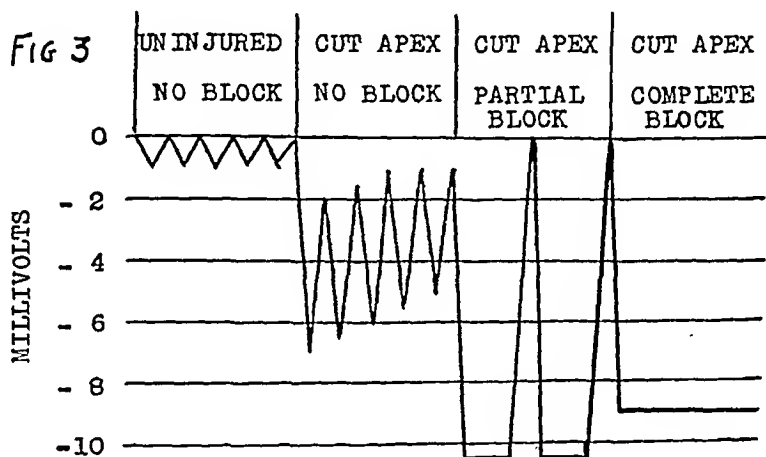
Fig. 3. D.C. potentials recorded from apex of frog heart before and after a cutting injury at this site, and before and after the induction of A-V block. Throughout the experiment the potential from an uninjured portion of the ventricle varied from −1 to −3 mv.

be correlated with the earlier electrographic observation of the development of a positive potential at the injured site in systole. That only a relatively and not an absolutely positive potential was thus observed during the moment of systole might be explained by the unduly long period of the measuring device.

4. When the beating of the ventricle was arrested by auricular-ventricular compression, a still larger and sustained negative potential was recorded with one electrode on the injured area. The average potential difference so recorded was 11.4 mv., with a range of from 3 mv. to 17 mv., in eleven experiments. The observed larger injury potential in the arrested heart as compared with the beating heart suggests that in the voltmeter observations the beating heart gives an incomplete picture of the disturbed electrical phenomena represented by the monophasic electrographic curve. Similar effects were noted in the arrested and injured heart which was mechanically stimulated to contract.

A graphic representation of an actual experiment showing the above phenomena is to be seen in figure 3.

COMMENT. The view that in the unipolar lead electrogram the location of the distal electrode is relatively unimportant for the character of the curve (6, 7, 8, 9) appears to apply also to the monophasic injury current (1, 2, and our results) and therefore supports the conclusion that the electrographic curve represents chiefly the potential changes at the electrode in contact with the injured region.

The actual reversal of potential from negative to positive during systole, as we obtained with bipolar and with unipolar leads (figs. 1 and 2), confirms the recent observations of Eyster (2), Ashman (17), and Katz (4), and together these findings argue strongly against the view that the electrical response in systole is attributable solely or primarily to potential changes in the uninjured region distant from the boundary of injury.

The microvoltmeter observations were entirely consistent with the electrographic findings. As was to be expected from the physical characteristics of the apparatus, the microvoltmeter proved unsuitable for following the relatively rapid changes of potential occurring in the normal heart muscle during systole. However, the electrical disturbance represented by the monophasic electrogram is of longer duration, and this was successfully followed by the microvoltmeter in direction but not accurately in magnitude or time values. Since our results were obtained when the "exploring" electrode was in contact with the injury, but not when it was in contact with uninjured tissue, it appears that the injured region exhibits important changes of potential represented by a monophasic electrogram, and that the monophasic record so obtained results largely, if not entirely, from changes occurring at the injured site. (This conclusion has no bearing on the question of whether the beginning, the duration, and the steepness of the initial ascent of the monophasic curve in bipolar leads are caused chiefly by changes of potential occurring at the electrode in contact with the injured or uninjured tissue.)

Such changes in potential occurring within the injured area do not necessarily indicate that the injured heart muscle cells are active. The deflection of the galvanometer could result from activity of the immediately adjacent tissue with suitable displacement of electrical charges thereby determined. This opinion appears to be in harmony with observations from Eyster's laboratory (12, 18). Moreover, monophasic curves have been obtained also from uninjured tissue (13, 14, 15, 16) when an area of normal tissue fails to respond to a stimulus which causes activity in other regions of the same myocardial unit. In the latter experiments the non-responding tissue does not manifest a negative injury potential at rest and the entire monophasic curve is positive.

A polarized double-ring of electrical charge limiting the injured tissue, as conceived by Eyster and his associates (2), gives a representation of the distribution of the electrical charges both at rest and during activity on the assumption that the electrical changes responsible for the monophasic injury curve occur in the muscle surrounding the injured area.

It remains to be seen whether the injury causes simply a partial (4, 16) or a complete depolarization of the injured surface, or also more profound electrical changes. A partial depolarization may explain the reversal of potential from

negative to positive during activity, but not the shifting to a positive value with reference to the uninjured resting tissue. For this to occur the action potential of the surface of the normal tissue should become negative with respect to the potential prevailing inside the resting cells. In fact, Curtis and Cole (19) have recently found that during the passage of an impulse the outside of the membrane of the squid giant axon acquires a negative potential with reference to the inside. Should these observations prove valid also for other excitable tissue, they might give a satisfactory explanation for the origin of the potential changes in injured heart muscle.

<center>CONCLUSIONS</center>

Evidence obtained both electrographically and by means of a suitable microvoltmeter indicates that the surface of an area of injury in the beating heart is the site of important changes in electrical activity during systole, and that the monophasic electrogram obtained from the injured heart results *predominantly* from potential changes at the electrode over the injured area. Our observations therefore stand in opposition to the traditional view in this regard, and they confirm the stand recently taken by several others (1, 2).

We wish to thank Dr. H. S. Burr and his associates in the Department of Anatomy for making available to us the microvoltmeter employed in this study.

<center>REFERENCES</center>

(1) WILSON, F. N., A. G. MacLEOD, F. D. JOHNSTON, AND I. G. W. HILL. Proc. Soc. Exper. Biol. and Med. 30: 797, 1933.
(2) EYSTER, J. A. E., W. J. MEEK, H. GOLDBERG AND W. E. GILSON. This Journal 124: 717, 1938.
(3) JOCHIM, K., L. N. KATZ AND W. MAYNE. This Journal 111: 177, 1935.
(4) SUGARMAN, H., L. N. KATZ, A. SANDERS AND K. JOCHIM. This Journal 130: 130, 1940.
(5) KATZ, L. N. Personal communication.
(6) WILSON, F. N., S. W. WISHART AND G. R. HERRMANN. Proc. Soc. Exper. Biol. and Med. 23: 276, 1925–26.
(7) CRAIB, W. H. The electrocardiogram, Special Report Series, Medical Research Council, London, 1930.
(8) WILSON, F. N., A. G. MacLEOD AND P. S. BARKER. Am. Heart J. 7: 207, 1931.
(9) EYSTER, J. A. E., F. MARESH AND M. R. KRASNO. This Journal 110: 422, 1934.
(10) BURR, H. S., C. T. LANE AND L. F. NIMS. Yale J. Biol. and Med. 9: 65, 1936.
(11) HOFFMANN, P. Handbuch der normalen und pathologischen Physiologie, vol. VIII/2 Energieumsatz. Berlin, Verlag von Julius Springer, 1928, p. 703.
(12) EYSTER, J. A. E., W. J. MEEK, H. GOLDBERG AND H. L. BARTSCH. This Journal 126: 488, 1939.
(13) SAMOJLOFF, A. Pflüger's Arch. 155: 471, 1914.
(14) SCHELLONG, F. Ztschr. f. d. ges. exper. Med. 86: 659, 1933.
(15) DE BOER, S. Cardiologia 2: 292, 1938.
(16) ASHMAN, R. AND N. WOODY. Proc. Soc. Exper. Biol. and Med. 42: 17, 1939.
(17) ASHMAN, R., W. S. WILDE AND N. WOODY. This Journal 129: 301, 1940.
(18) MEEK, W. J., J. A. E. EYSTER AND H. GOLDBERG. This Journal 126: 583, 1939.
(19) CURTIS, H. J. AND K. S. COLE. J. Cell. and Comp. Physiol. 19: 135, 1942.

THE DETERMINATION OF THE TRUE CELL VOLUME BY DYE DILUTION, BY PROTEIN DILUTION, AND WITH RADIOACTIVE IRON. THE ERROR OF THE CENTRIFUGE HEMATOCRIT

M. A. CHAPIN AND J. F. ROSS

From the Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine, Boston, Massachusetts

The relative volume of cells and plasma in the blood is usually determined by centrifugalization of a known volume of blood and by reading the volume of each component directly from a graduated hematocrit tube (1). The validity of this method rests upon the assumption that centrifugalization effects complete separation of cells from plasma, and that the shape of the cells is altered to the extent that they pack completely, i.e., with elimination of all intercellular space and exclusion of all plasma from among the cells (2). Millar (3) and Kennedy and Millikan (4), however, demonstrated that cells and plasma are not completely separated by the centrifugal force usually employed, but are separated completely only by a centrifugal force of a magnitude sufficient to produce cellular disintegration. Employing various techniques many investigators have demonstrated that the true cell volume is always less than the cell volume indicated by the centrifuge hematocrit method. The magnitude of this difference as reported has varied between 2 and 12 per cent.

We have found that the true cell volume may be determined accurately by a colorimetric method employing the dye T-1824 (Evans Blue). This method is simple, practical, and gives consistent results which we have verified by methods employing erythrocytes tagged with radioactive iron, and by determinations of plasma protein concentrations before and after dilution of blood with isotonic salt solution. These methods have shown the true hematocrit to be consistently 8.5 per cent less than the value indicated by the centrifuge hematocrit method.

METHODS. All blood used for these experiments (with the exception of the radioactively "tagged" cells) was drawn by venipuncture from normal human adults, care being exercised to avoid hemolysis. The dry oxalate mixture of Heller and Paul (5), which does not alter the erythrocyte volume (6), was used as an anticoagulant, 2 mgm. per ml. of blood being used uniformly.

The radioactive iron (Fe^{59}) was prepared by deuteron bombardment of iron phosphide in the Harvard cyclotron.[1] The separation and the technique of quantitative estimation of the radioactive iron have been described elsewhere (7, 8). The radioactively "tagged" iron was fed to a patient with hypochromic anemia, and after incorporation of the radioactive iron in newly formed hemoglobin and cells (9), venipuncture was performed and the blood collected in $2\frac{1}{2}$ per cent sodium citrate solution.

---

[1] We are indebted to Dr. B. R. Curtis and other members of the Harvard cyclotron crew for preparation of the radioactive iron.

The dye T-1824 (Evans Blue) (Eastman Kodak Co., lot no. 3873), in accurately weighed amount was dissolved in 0.85 per cent aqueous sodium chloride solution (isotonic salt solution) to give a concentration of 1.5000 mgm. per ml. of solution. Preparation and standardization of this solution was carried out as described by Gibson and Evans (10) using 8 different dilutions for accurate determination of the dye constant (K). The stock dye solution was diluted with isotonic salt solution to give a final concentration of 0.0600 mgm. per ml., a concentration suitable for use in the following experiments.

Colorimetric determinations of dye concentrations in plasma were performed on the Evelyn photoelectric colorimeter using the 620 and 540 filters as described by Gibson and Evelyn (11).

A size 2 International Equipment Co. centrifuge with a no. 240 head having an effective radius of 18 cm. was used. A relative centrifugal force (R.C.F.) of 1800 was obtained by centrifugalizing all hematocrits at 3000 r.p.m. (rate controlled with an attached tachometer) for 1 hour. Wintrobe tubes (1) were used, and duplicate or triplicate determinations were made on each blood sample. The readings in each instance checked within ± 0.2–0.3 per cent of the whole blood. All hematocrit readings cited include both the red cell column and the buffy coat, to allow direct comparison with the colorimetric and other methods of cell volume determination. This was deemed preferable to the practice of adding a constant value of 0.5 vol. per cent to the erythrocyte volume percentage (12), since the depth of the buffy coat varied considerably in different samples of blood.

Plasma protein determinations were performed according to the method of Kagan (13), calculations being based on an average of 4 determinations.

All volumetric glassware was recalibrated and chemically clean.

PROCEDURE AND EXPERIMENTAL RESULTS. The volume of cells was determined by 5 methods: 1. By determining the dilution of a known amount of dye added to a known amount of blood. 2. By determining the concentration of dye in the plasma of a blood-dye mixture, and then, by repeated washings of the cells with isotonic salt solution, removing all dye from the interstices of the cell mass, and colorimetrically determining the total dye present in the plasma and washings. 3. By determining the dilution of radioactively "tagged" erythrocytes added to a sample of blood. 4. By determining the dilution of plasma protein produced by mixing known volumes of blood and isotonic salt solution. 5. By centrifugalizing blood at an R.C.F. of 1800.

A. *The true cell volume as indicated by the dilution of a known amount of dye added to a blood sample.* The blue dye T-1824 is suited for use in determining the plasma volume since, when added to blood, it is distributed through the plasma but does not enter the cells (14). Quantitative recovery of the dye has consistently been obtained by washing the cells. Other notable properties of the dye, which perhaps contribute indirectly to this behavior, are that it rapidly combines with (or becomes firmly attached to) the globulin fraction of the plasma proteins, is separated from them only by rigorous procedures, and is not dialyzable through a collodion membrane (15). Thus, by determining the dilution of a

known amount of dye in a known volume of blood, the plasma volume and subsequently the cell volume can readily be determined by applying the equations:

Equation 1: Volume of plasma (ml.) =

$$\frac{\text{Mgm. dye added to the blood sample}}{\text{Concentration of dye in the plasma (mgm./ml.)}}$$

Equation 2: Volume of cells = volume of blood in sample − volume of plasma

The procedure and results of a typical experiment are given below:

Ten milliliters of oxalated whole blood were transferred to a centrifuge tube containing 1.00 ml. of dye solution (0.0600 mgm. of dye) and the two were thoroughly mixed by repeated

TABLE 1

SAMPLE	VOLUME OF BLOOD IN SAMPLE	VOLUME OF BLOOD AND ADDED DYE SOLUTION	CONCENTRATION DYE IN DILUTED PLASMA	VOLUME OF PLASMA AND VOLUME OF SALINE	CELL VOLUME PER CENT		TRUE CELL VOLUME LESS THAN CENTRIFUGE HEMATOCRIT	
					Determined colorimetrically	Determined by centrifugalization	Volume per cent	Per cent
	ml.	ml.	mgm./ml.	ml.				
A 1	10.00	11.00	0.00913	6.57	44.3	48.4		
2	10.00	11.00	0.00916	6.55	44.5	48.5		
3	10.00	11.00	0.00912	6.58	44.2	48.3		
					44.3	48.4	4.1	8.5
B 1	10.00	11.00	0.00886	6.77	42.3	46.3		
2	10.00	11.00	0.00888	6.76	42.4	46.5		
3	10.00	11.00	0.00881	6.73	42.7	46.6		
					42.5	46.5	4.0	8.6
C 1	10.00	11.00	0.00825	7.27	37.3	40.5		
2	10.00	11.00	0.00826	7.26	37.4	40.8		
3	10.00	11.00	0.00822	7.30	37.0	40.4		
					37.2	40.6	3.4	8.4

Comparison of the cell volume as determined by centrifugalization and by dye dilution.

inversions of the tube, care being exercised to avoid hemolysis. After a short period of centrifugalization the dyed plasma was removed and the dye concentration determined colorimetrically by comparison with dye-free plasma obtained from a mixture of 5.00 ml. of the same oxalated blood and 0.50 ml. isotonic salt solution (which was added to compensate for the saline in the dye solution). Having determined the plasma dye concentration, and knowing the total amount of dye added to the blood, the volumes of plasma and cells present in the blood sample were calculated by application of equations 1 and 2.

The consistent results obtained with the colorimetric procedure and the constant relationship which these results bear to the centrifuge hematocrit determinations are illustrated in table 1, in which separate determinations on three aliquots of three separate blood samples are tabulated. The values obtained by the dye method agree within the limits of accuracy of the galvanometer readings of the colorimeter (1 per cent), and in each instance are approximately 8.5 per cent lower than the centrifuge hematocrit.[2]

[2] Volume per cent difference should not be confused with per cent difference.

B. *The cell volume as indicated by the concentration of dye in the plasma of a blood-dye mixture and the recovery of dye from the sample.* This method differs from the first only in the method of determining the total amount of dye which is present in the sample, but substantiates the observations of Gregersen and Shiro (14) that all dye can be recovered from the cells by repeated washings, and indicates that significant amounts of dye do not pass into either erythrocytes or leucocytes. It is applicable to samples of blood in which the exact dye content is not known (e.g., samples withdrawn from patients during plasma volume determinations with the dye method). A typical experiment and the results obtained are described:

Oxalated blood (10.00 ml.) was thoroughly mixed with 1.00 ml. of dye solution (0.0600 mgm. of dye) and after centrifugalization the plasma was pipetted off and placed in an accurately graduated container. The plasma dye concentration was determined as in procedure A, and all plasma was saved (this is feasible when the micro-unit of the colorimeter is used). Isotonic salt solution (3.00 ml.) was then mixed with the cells and after centrifugalization the supernatant fluid was removed and added to the original plasma. Three such washings were carried out, the wash fluids being saved with the plasma. The final washing was free from visible evidence of dye content.

The total volume of combined washings and plasma was then read from the graduated container, and the concentration of dye determined colorimetrically by comparison with dye-free plasma proportionately diluted with isotonic salt solution. The total dye originally present in the blood sample was then calculated, and equations 1 and 2 applied to determine the plasma and cell volume.

The accuracy with which the cell volume may be determined by this method is shown in table 2, and the results are compared with those obtained by the method outlined in procedure A and with those obtained by centrifugalization.

Gregersen and Shiro (14) demonstrated that when blood samples containing either the dye T-1824 or Brilliant Vital Red were centrifugalized, an average of 4.2 per cent (ranging from 2.3 to 7.0 per cent) of dye (and plasma) remained in the cell mass. This is not to be interpreted as indicating that the true cell volume is 4.2 per cent lower than the centrifuge hematocrit, as has been done by Stead and Ebert (16) and Shohl and Hunter (12). As shown in table 3 the per cent of plasma retained in the cell mass does not represent the percentage difference between the actual hematocrit and the centrifuge hematocrit. Thus the inclusion of 5.7 per cent of the plasma in the cell mass produces an error of approximately 8.3 per cent in the centrifuge hematocrit.

C. *Determination of the true cell volume by dilution of radioactively "tagged" erythrocytes and dye.* The radioactive isotope of iron (Fe^{59}) when fed to anemic individuals is incorporated in newly formed hemoglobin within the erythrocytes (9), where it remains until the disintegration of the cell (17). Appropriate techniques allow the quantitative detection of this material, and by adding erythrocytes containing a known amount of radioactivity to a sample of blood, the exact blood volume can be determined from calculations of the dilution of the radioactivity. Subsequent calculations, based on dye dilution, will indicate the plasma volume and the cell volume.

Citrated blood (49.98 ml.) containing erythrocytes "tagged" with radioactive iron was pipetted into a 250.00 ml. volumetric flask. The total radioactivity[3] of this blood (as indicated by measurements performed on other aliquots of the same blood) amounted to 681.5 counts per minute. Dye solution (1.00 ml.) containing 1.5000 mgm. of T-1824 was added and the volume made to 250.00 ml. with normal compatible blood. Complete mixing was effected without hemolysis, and 5 aliquots (20.00 ml. each) were removed and their content of radioac-

TABLE 2

SAMPLE	CELL VOLUME PER CENT DETERMINED BY		
	Centrifugalization	Dye dilution	Dye recovery
1	43.6	39.8	39.8
2	47.7	43.7	43.8
3	42.6	38.7	39.2
4	49.6	45.4	45.5
5	40.9	36.4	36.2
6	44.0	40.8	40.8

Cell volume per cent determined by centrifugalization, dye dilution and dye recovery.

TABLE 3

	VOLUME OF SAMPLE	DYE SOLUTION ADDED	DILUTED PLASMA ABOVE CELLS	CONCENTRATION DYE IN DILUTED PLASMA	DYE IN PLASMA		Among cells	TRUE PLASMA VOLUME	CELL VOLUME PER CENT DETERMINED BY		TRUE CELL VOLUME LESS THAN CENTRIFUGE HEMATOCRIT	
					Above cells				Centrifuge	Dye	Volume per cent	Per cent
	ml.	ml.*	ml.	mgm./ml.	mgm.	per cent	per cent	per cent				
1	10.0	1.0	6.10	0.00925	0.0564	94.0	6.0	54.9	49.0	45.1	3.9	8.1
2	10.0	1.0	6.64	0.00857	0.0569	94.8	5.2	60.0	43.6	40.0	3.6	8.3
3	10.0	1.0	6.23	0.00905	0.0564	94.0	6.0	56.3	47.7	43.7	4.0	8.4
4	10.0	1.0	6.74	0.00844	0.0569	94.8	5.2	61.0	42.6	39.0	3.6	8.5
5	10.0	1.0	6.04	0.00932	0.0563	93.8	6.2	54.4	49.6	45.6	4.0	8.1
Average........................							5.7					8.3

* 0.0600 mgm. dye T-1824 per ml.

The amount of dye and plasma remaining in the cell mass and comparison of the cell volume determined by dye dilution and centrifugalization.

tively "tagged" cells determined. The radioactivity was found to be 2.7 counts per minute per ml. of blood-dye mixture, indicating the total blood volume to be 252.4 ml. (an error of 0.9 per cent).

The plasma volume, determined as in the preceding experiments, was found to be 167.5 ml. The cell volume was, therefore, 84.9 ml. or 33.6 volumes per cent of the blood sample. The average of 4 centrifuge hematocrit values was 36.7 volumes per cent. The true cell volume was thus 8.5 per cent lower than the centrifuge hematocrit.

[3] Radioactivity reported in counts per minute on a Geiger-Müller counter.

D. *Determination of the true cell volume by measurement of plasma protein dilution.* The concentration of protein per unit volume of plasma may be determined before and after addition of a known amount of isotonic salt solution to a blood sample of known volume, and the volume of plasma in the sample calculated from the equation:

Equation 3:

$$X = \frac{VP_2}{P_1 - P_2}$$

When X = Volume of plasma in the blood sample

V = volume of isotonic salt solution added to sample

P_1 = concentration of protein, in mgm./ml. before dilution

P_2 = concentration of protein, in mgm./ml. after dilution

The volume of cells in the sample is then calculated from equation 2.

For example, the plasma protein concentration of an undiluted sample of blood was 73.50 mgm. per ml. After dilution of 10.00 ml. of blood with 1.00 ml. isotonic salt solution, the concentration was 62.00 mgm. per ml. Applying equation 3:

$$\text{Volume of plasma in milliliters} = \frac{1.00 \text{ ml.} \times 62.00 \text{ mgm./ml.}}{73.50 \text{ mgm./ml.} - 62.00 \text{ mgm./ml.}} = 5.39 \text{ ml.}$$

From equation 2, the volume of cells = 4.61 ml.

$$\text{Cell volume} = 46.1 \text{ per cent}$$

Application of the dye dilution method to the same sample of blood showed a true cell volume of 46.5 per cent. The average of 2 centrifuge hematocrits for the blood was 50.6 per cent.

This method serves as a check on the validity of the dye dilution method for determining the cell volume. The results obtained by the two methods agree very closely, and both are more than 8 per cent lower than the centrifuge hematocrit.

E. *The effect of variations in the absolute cell volume on the packing of cells.* To determine the effect of cell volume variations on separation of cells from plasma by centrifugalization, the relative volume of cells was adjusted in 7 aliquots of an oxalated blood sample by removing plasma from some and adding this same plasma to others. In this fashion, the centrifuge hematocrit was caused to range from 20.9 to 79.9 per cent. The cell volume of each sample was determined by the dye dilution methods described in procedure A and by centrifugalization. The results are shown in table 4.

Comparison of the cell volumes determined by the 2 methods indicates that the amount of plasma remaining in the cell mass after centrifugalization varies directly with the volume of the cell mass; hence, the relationship of the true cell volume to that indicated by centrifugalization is constant.

F. *The cell volume determined by the colorimetric and centrifuge hematocrit methods in the blood of different human subjects.* The cell volume of blood from numerous human subjects was determined by centrifugalization and by dye dilution

methods to ascertain whether or not the variable factors existing in different bloods (pigments, proteins, etc.) influenced the difference indicated by the two procedures.   Table 5 lists unselected determinations on blood samples from 10 different human subjects.   It is evident that the true cell volume is consistently some 8.5 per cent lower than the centrifuge hematocrit value.

DISCUSSION.   The concept that separation of cells from plasma is complete when continued centrifugalization at a given rate produces no further packing of

TABLE 4

SAMPLE	CELL VOLUME PER CENT		CELL VOLUME		PER CENT TRAPPED PLASMA
	Determined by centrifugalization	Determined colorimetrically	Volume per cent less	Per cent less	
1	20.9	19.1	1.8	8.6	2.2
2	29.9	27.3	2.6	8.7	3.6
3	48.4	44.3	4.1	8.5	7.4
4	53.4	48.7	4.7	8.8	9.2
5	59.7	54.7	5.0	8.4	11.0
6	68.4	62.5	5.9	8.6	15.7
7	79.7	72.7	7.0	8.8	25.6

Comparison of the cell volume determined by centrifugalization and by dye dilution in aliquots of blood with varying relative cell volumes.

TABLE 5

SAMPLE	CELL VOLUME PER CENT		VOLUME PER CENT LESS	PER CENT LESS	PER CENT TRAPPED PLASMA
	Determined by centrifugalization	Determined colorimetrically			
1	40.3	37.1	3.2	8.0	5.1
2	42.6	38.7	3.9	9.1	6.3
3	43.6	39.8	3.8	8.7	6.3
4	44.0	40.2	3.8	8.6	6.4
5	47.5	43.4	4.1	8.6	7.2
6	47.7	43.5	4.2	8.8	7.4
7	48.4	44.3	4.1	8.5	7.4
8	49.0	45.1	3.9	8.0	7.1
9	49.6	45.4	4.2	8.5	7.7
10	53.4	48.7	4.7	8.8	9.2

Comparison of cell volume values obtained by the colorimetric and centrifuge hematocrit methods on the blood of 10 normal human subjects.

the cell layer is erroneous.   Application of an R.C.F. of 1800 (usually employed in hematocrit determinations) for 1 hour can be expected to effect only 91 to 92 per cent complete separation (3).

Employing the simple procedure of dye dilution, we have demonstrated that the true cell volume is consistently lower than the centrifuge hematocrit value (determined at an R.C.F. of 1800 for 1 hour).   The validity of this method is based upon the assumption that none of the dye enters the cells.   The quantita-

tive recovery of all dye by repeated washing of the cell mass, and the identical values for cell volume obtained by the plasma protein dilution method serve to justify this assumption, and establish the values so obtained as the true cell volume.

The quite constant relationship between the true cell volume percentage and the centrifuge hematocrit in different subjects and over a wide range of cell volume values suggests that a correction factor might successfully be applied to centrifuge hematocrits to determine the true values. We are reluctant to advise such a procedure, however, since the degree of packing of the cell layer by centrifugalization may be influenced by extreme variations of the erythrocyte size, or by very marked changes in the specific gravity or viscosity of the plasma.

The error of the centrifuge hematocrit is large enough to influence materially the values of certain procedures which are dependent on the absolute cell volume percentage for accuracy. Thus, a positive error of 8.5 per cent in the hematocrit renders the total cell volume, as calculated according to the Keith, Rowntree and Geraghty procedure (18), some 15 to 16 per cent too high. The actual volume of the individual erythrocyte is less than commonly assumed, and calculations of electrolyte content of erythrocytes based upon analysis of packed cells are probably in error (19, 20). In routine hematological procedures in which only *comparative* values for the hematocrit are necessary, the centrifuge hematocrit is satisfactory provided that determinations are made under identical physical conditions.

The value for the true cell volume percentage as determined by us is significantly lower than that reported recently by Shohl and Hunter (12). The different values and wide range of discrepancy (1–6 per cent) between the true cell volume and the centrifuge hematocrit reported by these investigators may be due to their use of small volumes of blood and large amounts of dye.

SUMMARY

1. Centrifugalization of blood as usually performed does not effect complete separation of cells and plasma, and gives a value for the cell volume which is too high.

2. The true cell volume can be determined by dilution of the dye T-1824, and by dilution of plasma protein. The percentage of cells so determined is consistently some 8.5 per cent lower than the centrifuge hematocrit value.

3. The dye T-1824 does not enter into or adhere to the cellular elements in the blood in significant amounts, and quantitative recovery is possible.

4. A technique for determination of the true cell volume percentage in blood samples removed during *in vivo* plasma volume determinations is described.

5. Use of radioactively "tagged" erythrocytes can be employed for accurate determination of blood volume.

6. The significance of the error introduced in certain procedures by the erroneously high centrifuge hematocrit value is discussed.

REFERENCES

(1) WINTROBE, M. M.   J. Lab. and Clin. Med. **15**: 287, 1929.

(2) HIROTA, K.   J. Biophysics (Japan) **1**: 233, 1925; **2**: 283, 1927.

(3) MILLAR, W. G.   Quart. J. Exper. Physiol. **15**: 187, 1925.

(4) KENNEDY, J. A. AND G. A. MILLIKAN.   J. Physiol. **93**: 276, 1938.

(5) HELLER, V. G. AND H. PAUL.   J. Lab. and Clin. Med. **19**: 777, 1933.

(6) HAM, T. H. AND F. C. CURTIS.   Medicine **17**: 447, 1938.

(7) CHAPIN, M. A. AND J. F. ROSS.   Communication submitted for publication.

(8) ROSS, J. F. AND M. A. CHAPIN.   Rev. Scientific Instruments **13**: 77, 1942.

(9) ROSS, J. F. AND M. A. CHAPIN.   J. Clin. Investigation **20**: 437, 1941.

(10) GIBSON, J. G., II AND W. A. EVANS.   J. Clin. Investigation **16**: 301, 1937.

(11) GIBSON, J. G., II AND K. A. EVELYN.   J. Clin. Investigation **17**: 153, 1938.

(12) SHOHL, A. L. AND T. H. HUNTER.   J. Lab. and Clin. Med. **26**: 1829, 1941.

(13) KAGAN, B. M.   J. Clin. Investigation **17**: 373, 1938.

(14) GREGERSEN, M. I. AND H. SHIRO.   This Journal **121**: 284, 1938.

(15) Personal observations.

(16) STEAD, E. A. AND R. V. EBERT.   This Journal **132**: 411, 1941.

(17) HAHN, P. F., W. F. BALE, J. F. ROSS, R. A. HETTIG AND G. H. WHIPPLE.   Science **92**: 131, 1940.

(18) KEITH, N. M., L. G. ROWNTREE AND J. T. GERAGHTY.   Arch. Int. Med. **16**: 547, 1915.

(19) OBERST, F. W.   J. Biol. Chem. **108**: 153, 1935.

(20) BUTLER, A. M. AND E. M. MACKAY.   J. Biol. Chem. **106**: 107, 1934.

THE EFFECT OF NICOTINE ON UTERINE RESPONSES TO HYPOGASTRIC NERVE STIMULATION

DONAL SHEEHAN AND JOHN S. LABATE

From the Department of Anatomy, New York University, College of Medicine

Nicotine injected intravenously acts on autonomic ganglia, and in suitable doses blocks at this site the further transmission of nerve impulses, without affecting postganglionic fibers. It has been used, therefore, to locate the precise synaptic connections along specific autonomic pathways.

Sympathetic fibers reach the uterus along the hypogastric nerve, stimulation of which gives rise in the rabbit, monkey and pregnant cat, to tetanic contraction of the uterus followed by a period of inhibition (1, 2). Langley and Anderson (1) observed marked reduction, and in some instances abolition, of these responses in the cat and rabbit following intravenous injection of nicotine, and they concluded therefore that the majority of the synaptic connections along this nerve pathway lie peripheral to the inferior mesenteric plexus. The hypogastric nerve would then convey preganglionic fibers to the uterus. Their observations were confirmed by Cushny (3), but recently Sherif (4) has reported that, in the bitch, nicotine tartrate injected intravenously had no effect on uterine responses to hypogastric nerve stimulation, and has concluded that the hypogastric nerves consist mainly of postganglionic fibers. The contradiction in experimental evidence remains unexplained. The question has therefore again been taken up as part of an investigation of the autonomic pathways to the uterus in the monkey.

METHOD. Nine experiments were carried out, seven in immature female monkeys (Macaca mulatta) and two in bitches. The animals were anesthetized with nembutal, injected intraperitoneally, and prepared for recording uterine movements in response to hypogastric nerve stimulation, as described in a previous paper (2). Nicotine alkaloid in 2 per cent solution was administered intravenously in doses of 10 mgm. at a time. A total of 40 mgm. was given in some experiments.

RESULTS. In all experiments in the monkey nicotine in sufficient dosage abolished the motor phase of nerve stimulation but did not affect the inhibitory response. The dose of nicotine varied in different animals. In one monkey 10 mgm. abolished the excitatory response; in another 40 mgm. had to be given before the motor response to nerve stimulation entirely disappeared, although it was much diminished after an initial dose of 10 mgm. Two hours after the administration of nicotine, stimulation of the hypogastric nerves once more gave excitation of uterine activity, and this was again abolished by a further dose of nicotine.

In the bitch the initial excitatory response to hypogastric nerve stimulation was never as marked as in the monkey. However, the results with nicotine in

these animals were identical with those obtained in the monkey in that, following nicotine (20 mgm.), the excitatory effect of hypogastric nerve stimulation was completely abolished.

DISCUSSION. The weight of experimental evidence can only be interpreted by concluding that the fibers in the hypogastric nerves, stimulation of which results in a motor response in the uterus of the monkey and bitch, are preganglionic in nature. Histological evidence in support of such a conclusion has been offered by Kuntz and Moseley (5). Sherif's negative findings (4) may have been due to a relatively inactive preparation of nicotine; certainly the high dosage which he used—up to 80 mgm. in some experiments—is well within the lethal range.

The persistence of the inhibitory phase of the response has not been previously emphasized. It suggests that the synaptic connections along the
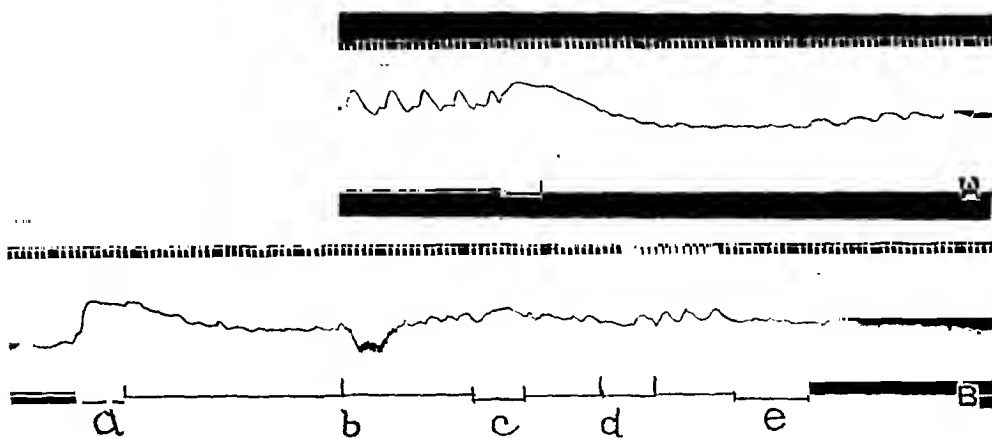
Fig. 1. A. Uterine response to hypogastric stimulation in the monkey; motor and inhibitory phases. B. The same. *a*, hypogastric nerve stimulation; *b*, intravenous injection 10 mgm. nicotine; *c*, hypogastric nerve stimulation; *d*, intravenous injection 10 mgm. nicotine; *e*, hypogastric nerve stimulation.

inhibitory pathway may lie above the point of stimulation, and the hypogastric nerve would then contain in addition some postganglionic fibers to the uterus.

The location along the *motor* pathway to the uterus of synaptic connections close to the organ of supply, follows the pattern which is associated with the parasympathetic rather than with the sympathetic branch of the autonomic nervous system. There is experimental evidence to support the contention that some of the hypogastric nerve fibers to the uterus are cholinergic in nature. Sherif (4) found that the concentration of acetylcholine in the blood in the uterine vein of a bitch was increased by stimulation of the hypogastric nerves and that the contraction of the uterus produced thereby was accentuated by the injection of eserine. The influence of cocaine (2) on the uterine reactions induced by hypogastric nerve stimulation also indicated that in the rabbit and monkey these nerves contain a relatively higher percentage of cholinergic elements.

SUMMARY

In the uterus of the monkey and of the bitch nicotine in suitable doses abolished the motor effect of hypogastric nerve stimulation.

The inhibitory phase in the monkey was not affected by nicotine.

The evidence suggests that the hypogastric nerves in the monkey contain preganglionic motor fibers and postganglionic inhibitory fibers to the uterine musculature, and in the bitch preganglionic motor fibers.

The motor fibers to the uterus contained in the hypogastric nerves of the monkey and bitch are probably cholinergic in nature.

REFERENCES

(1) LANGLEY, J. N. AND H. K. ANDERSON.   J. Physiol. 19: 122, 1895.
(2) LABATE, J. S.   J. Pharmacol. and Exper. Therap. 72: 370, 1941.
(3) CUSHNY, A. R.   J. Physiol. 35: 1, 1906-07.
(4) SHERIF, M. A. F.   J. Physiol. 35: 298, 1935.
(5) KUNTZ, A. AND R. L. MOSELEY.   J. Comp. Neurol. 64: 63, 1936.

FACTORS CONCERNED IN THE DEVELOPMENT OF TETANY BY THE RAT[1,2]

DAVID M. GREENBERG, MURIEL D. D. BOELTER AND BENJAMIN W. KNOPF

From the Division of Biochemistry, University of California Medical School, Berkeley

Little is known about the mechanism concerned in the onset of the syndrome of tetany. No single etiological factor is involved and no alteration in any single constituent of the body has been found which occurs invariably in tetany.

According to Dragstedt (2) the most constant change in tetany is the increased excitability of the peripheral nerves to electrical stimulation. This indicates that the nerve endings are the points primarily affected in this condition. In support of this view, Hartridge and West (3) found that in thyro-parathyroidectomized dogs attacks of tetany could be abolished by sub-paralytic doses of curare. West (4) also observed that all three forms of tetany, tonic, clonic and fibrillary, can occur from 6 to 36 days after mid-dorsal transection of the spinal cords of thyro-parathyroidectomized dogs.

Contrary to the above, Noël-Paton, Findlay and Watson (5) came to the conclusion that in the tetany following thyro-parathyroidectomy, all the nervous symptoms were due to the condition of the central nervous system. Section of the nerves abolished all the spasms, tremors and jerkings of the muscles supplied, whereas transection of the spinal cord abolished the tonic and clonic spasms but not the tremors and jerkings. In tetany due to magnesium deficiency, Greenberg and Tufts (6) were not able to abolish the convulsions with sub-paralytic doses of curare.

Most of the physiological studies on tetany have been carried out on the thyro-parathyroidectomized dog. Since the characteristics of tetany vary in different animal species, it is desirable to carry out comparative studies on other species and among these the rat is one of the most convenient. It is also desirable to determine and compare the characteristics of tetany as produced by the various known methods. In the present work the characteristics of tetany in the rat as produced by a variety of means have been studied. By experiments involving administration of barbiturates and curare and by spinal transection, evidence has been obtained that the integrity of the central nervous system at a level higher than the spinal cord is necessary for the development of the convulsions of tetany in the rat. In a previous work, Greenberg and Tufts (6) through the difference in the pharmacological effect of certain drugs, concluded that the midbrain or the pons is in some manner involved in the tetany of magnesium deficiency.

METHODS OF STUDY.   Tetany was produced by dietary magnesium deficiency, dietary calcium and vitamin D deficiency, fasting of rachitic rats, parathyroidectomy and thyro-parathyroidectomy with dietary calcium deficiency.

Two stimuli both of a qualitative nature were employed to determine whether an animal was hyperirritable and thus prone to suffer from convulsions of tetany. One stimulus was the hissing sound of an air jet.   The other was a mild galvanic shock from an induction coil.   In the latter test the rat was placed on a wood plate covered with alternating strips of brass contained on the bottom of a deep glass jar, which was connected by wires to the induction coil.   The current could be turned on and off by a key as desired.

RESULTS.   *The convulsions of tetany in the rat.*   The motor manifestations of tetany in the dog as described by West (4) are 1, *tonic*, where continued contraction of a muscle or a group of muscles produces a strongly marked posture; 2, *clonic*, where whole muscles, or a large portion of the fibers of a muscle contract nearly synchronously, producing jerks and twitches; and 3, *fibrillary*, consisting of a rapid repeated contraction of a small group of fibers in one or more areas.   Bryan and Garrey (7) observed that tetany in the thyro-parathyroidectomized dog is initiated by a rise in body temperature and overventilation due to panting.

A detailed description of the characteristic features of the convulsions of tetany in the rat as produced by magnesium deficiency has been given by Kruse, Orent and McCollum (8).   In an attack, the animal races in a circle until it falls on its side unconscious.   The entire body becomes rigid, with head stretched back, fore limbs extended at the upper joints and flexed at the metacarpophalangeal joint, and hind limbs extended backward.   The jaws are clenched and all respiratory movements cease during the attack to return only with relaxation of the musculature.   This tonic epileptiform convulsion was the most characteristic feature observed in the present study by all the methods of inducing tetany that were employed.

During the convulsions regurgitation of the stomach contents and the appearance of a foam about the mouth is a common feature.

Comparison of Tetany Produced by Different Regimens.   Table 1 gives a summary of the incidence of convulsive attacks, both spontaneous and induced, on the different regimens that were employed.   On all of the regimens the characteristic features of the convulsions were the same and followed essentially the pattern given above.   When a convulsion was induced by a galvanic stimulus, the susceptible animal fell over on its side and immediately went into a tonic spasm in which the body became rigid, with both fore and hind limbs extended and the fore paws clenched.

The severity of the tetany is approximately indicated by the number of deaths. Deaths following observed attacks of tetany are indicated in table 1.   In most instances, however, the animals were found dead in their cage in the morning. While convulsions probably preceded death in these cases, there is no assurance of it.   The highest death rate occurred in the tetany of magnesium deficiency followed by that of the calcium deficient thyro-parathyroidectomized rats.

The relative susceptibility to the two stimuli that were used is also interesting. In magnesium deficiency, as was more conclusively demonstrated in a previous paper (6), the hissing sound of an air blast is highly effective and the galvanic shock almost always ineffective.

Table 1 shows that the animals on the low-calcium-low-D regimen and the fasting rachitic animals responded only to the galvanic stimulus and not at all to sound.   The parathyroidectomized rats on the low calcium diet responded to

TABLE 1

Summary of the incidence of tetany on various regimens

REGIMEN	ANIMALS, NO.	TIME	OBSERVATIONS OF SPONTANEOUS CONVULSIONS, NO.	GALVANIC STIMULUS		SOUND STIMULUS		DEATHS ALL CAUSES, NO.	PARALYSIS	
				Tests, no.	Convulsions, no.	Tests, no.	Convulsions, no.		Spontaneous, no.	After galv. shock, no.
Magnesium deficiency*...	35	4–9 wks.	6	4	1	30	13	32**	0	0
Calcium and vitamin D deficiency....	14	3–7 wks.	0	19	6	29	0	4***	1	5
Fasting rickets..........	14	4–48 hrs.	0	34	11	19	0	2		1
Parathyroidectomy and low calcium diet.......	33	3–13 wks.	1	31	5	22	1	14†	10	17
Thyro-parathyroidectomy and low calcium diet..................	52	2–8 wks.	9	99	25	122	59	38‡	3	7
Sodium phosphate injection††	4	15 mins.	2	1	0	1	1	4‡‡		
Sodium citrate injection§..................	8	15 mins.	2	2	2	3	0	4¶		

* Included 8 animals on the same diet with added 10 mgm. ascorbic acid per 100 grams food.

** Eight deaths after observed attack of tetany.

*** Two deaths after observed attack of tetany, 2 deaths after paralysis.

† One death after observed attack of tetany.

‡ Eleven deaths after observed attack of tetany.

†† Dose 5 ml. 10 per cent Na_2HPO_4 per 100 grams body weight injected intraperitoneally.

‡‡ Two deaths immediately following attack of tetany.

§ Dose 1 ml. 1 per cent sodium citrate per 100 grams body weight injected intraperitoneally.

¶ All deaths immediately following attack of tetany.

a higher degree to the galvanic shock than to sound while the thyro-parathyroidectomized animals responded readily to both stimuli but with the sound stimulus being nearly twice as effective.

A brief account of the salient features on each regimen is given below.

Magnesium deficiency.   The results of an extensive study of the tetany due to this condition have been reported in a previous work (6).   It was there pointed out that the best stimulus for inducing the convulsions of tetany was the hissing sound of an air blast and that a galvanic shock from an induction coil was seldom

effective.   In the 35 animals of the present study, a group of 8 was reared on the magnesium deficient diet but with the addition of crystalline ascorbic acid. There was no noticeable difference in response due to the ascorbic acid.

Calcium and vitamin D deficiency.   A diet lacking calcium only is not sufficient to produce tetany in the rat even though the blood serum calcium concentration is greatly lowered (9).   Tetany results, however, if the diet lacks vitamin D as well as calcium.   Weaned rats (28 days old) became subject to the convulsions of tetany after being 6 to 7 weeks on this regimen.

No spontaneous seizures were observed but convulsions were produced in about one-third of the animals subjected to galvanic shock.   The sound stimulus was completely ineffective.   The characteristics of the convulsions were quite like those observed in the tetany of magnesium deficiency.

With the passage of time the galvanic shock caused paralysis and prostration such as is found in uncomplicated calcium deficiency (9) rather than attacks of tetany.

The animals on this regimen scarcely grew at all.   The food consumption which was initially 5 grams per rat per day fell to as low as 3 grams per rat per day after 5 weeks.   Analysis of the blood showed that the serum calcium was very low (6.3 mgm. per 100 ml.) and the serum phosphorus about normal (11.4 mgm. per 100 ml.).

Templin and Steenbock (10) fed adult female rats a diet low in calcium (56 mgm. per 100 grams food) and lacking in vitamin D for eight months, during which time no attacks of tetany were observed, although the concentration of serum calcium fell as low as 4.8 mgm. per 100 ml.   These animals, however, were not subjected to electrical stimulation.   Jones and Cohn (11) have noted that vitamin D protects the rat to a certain extent from the effects of calcium deficiency.   The serum calcium was not as low and the mortality not as high when additional vitamin D was administered.

Fasting rickets.   It has been observed that rachitic rats placed on a favorable healing diet or if simply starved, may succumb to attacks of tetany (11–13). In the present study 14 severely rachitic rats[3] were shifted to our stock colony diet but they refused to eat.   They were tested by galvanic and sound stimulus from 4 to 48 hours after this dietary change with the results shown in table 1.

Chemical analysis of the blood of these rats showed that the serum calcium concentration was lowered (average, 7.2 mgm. per 100 ml.) and the serum inorganic phosphorus was normal (11.5 mgm. per 100 ml.).

It is to be noted from the results on the low calcium-low vitamin D and the fasting rachitic animals, that when the tetany was associated with lack of vitamin, only electric stimulation was effective and the sound stimulus completely ineffective in producing attacks of tetany.

Parathyroidectomy and low calcium diet.   The rat is resistant to the onset of tetany following removal of the parathyroid or thyro-parathyroid glands if the diet is adequate (14).   However, tetany develops if the dietary calcium is low (15), or the phosphorus is high (16).

---

[3] Kindly supplied by Mr. Theodore Sanford of the Booth Laboratories, Oakland.

In the present study the two visible superficial parathyroids that occur in the rat were removed from a group of 33 rats which then were maintained on our standard low calcium diet (9). Removal of the parathyroids alone was comparatively ineffective in producing tetany even on the low calcium diet. The results are shown in table 1. The electric stimulus was more effective than the sound stimulus in evoking convulsions in this condition. The greatest liability to tetany occurred between 3 and 8 weeks after the operation.

With the progress of time, the parathyroidectomized animals became more subject to paralysis and prostration than to tetany. Ten animals became paralyzed spontaneously and 17 succumbed to this condition after being subjected to the galvanic current. Of the 14 animals that died, the greater number did so while in a paralytic condition.

The serum calcium concentration was at its lowest shortly after removal of the parathyroids and then tended to increase. Two weeks after the operation the calcium concentration was 5.3, at 4 weeks 7.3, and 8 weeks 9.5 mgm. per 100 ml. serum.

Incomplete removal of all parathyroid tissue may account for the low incidence of tetany in the parathyroidectomized animals. Hoskins and Chandler (17, 18) and Overholser (19), contrary to the opinion of earlier workers, found an incidence of accessory parathyroids in only 5 to 7 per cent of the rats upon careful examination by serial sections of the neck region anterior to the vertebrae. Occasionally, however, strains of rats were found in which accessory parathyroids increase to the extent of 20 per cent.

Thyro-parathyroidectomy and low calcium diet. In the thyro-parathyroidectomized animals on the low calcium diet, the incidence of tetany was high and the attacks of tetany were severe. Sufficient desiccated thyroid to supply an adequate amount of hormone was added to the diet. Consequently a deficiency of thyroid hormone was not involved in the increased incidence and severity of the tetany. If the rarity of accessory parathyroid tissue in the rat is true, it is difficult to explain the differences in the results of the two regimens.

Thyro-parathyroidectomy was performed on 52 animals when they were about 5 weeks of age and then they were placed on the low calcium diet with added desiccated thyroid.[4] The animals exhibited hardly any growth and very few animals were able to survive for as long as two months after the operation.

The thyro-parathyroidectomized animals become nervous, hyperexcitable and very active. As shown in table 1, they are easily susceptible to tetany. The sound stimulus proved somewhat more effective in inducing convulsions than the electric current. The time of greatest susceptibility was between 3 to 5 weeks after the operation. The severity of the condition is shown by the fact that 11 deaths occurred after observed attacks of tetany. It seems likely that many of the other 27 animals that died also succumbed following spontane-

---

[4] We have noted recently that an even more efficient method of producing tetany is to maintain the rats for about 5 weeks on the low calcium diet and then thyro-parathyroidectomize.

ous attacks during the night.   The blood serum calcium quickly dropped and remained at a low level of concentration, ranging between 5 and 7 mgm. per 100 ml. of serum.   This is in marked contrast to the rats in which only the parathyroids were removed.   In this group the serum calcium at first dropped and then rose again to normal.

In the course of time some of the features associated with pure calcium deficiency developed in the animals.   There were 3 spontaneous and 7 induced cases of paralysis and prostration.

Di-sodium phosphate and sodium citrate injection.   A few experiments were carried out on the tetany produced by injections of these salts into normal rats in order to observe how closely this compared with the tetany caused by the deficiency regimens given above.

The dosage employed is given in table 1.   The first effect of the injection of either salt was to make the animals apathetic and stuporous.   Apparently they were only semi-conscious.   The convulsions of tetany showed the same general features, i.e., the tonic positional spasm described above.   Besides this there was a great deal of clonic contraction not commonly observed on the other regimens.   Spontaneous convulsions set in about 10 to 15 minutes after injection of the di-sodium phosphate which eventually ended in death.   Convulsive seizures could also be induced prior to their spontaneous onset by either the air blast or galvanic shock.

In the sodium citrate injected animals, convulsions could be elicited in from 5 minutes to 1 hour after the injection.   Seizures were produced by galvanic stimulation and by touching and pinching the animals, but not by the hissing sound of the air blast.   Two out of eight animals had spontaneous attacks.   The condition appeared to be less severe in the citrate injected than in the phosphate injected animals.

The Rôle of the Central Nervous System in Tetany.   The involvement of the central nervous system in the syndrome of tetany, at a level higher than the spinal cord, is demonstrated by the experimental results described below.

Barbiturates.   Noël Paton and Findlay (20) noted that ether anesthesia completely stopped attacks of tetany in thyro-parathyroidectomized dogs.   Administration of barbiturates will prevent the onset of tetany in susceptible rats.   Only sedative, not anesthetic doses are required.   It was previously shown that doses of $\frac{1}{3}$ to $\frac{1}{2}$ the anesthetic level of sodium amytal prevented the onset of the convulsions of tetany in magnesium deficient rats when they were subjected to the hissing sound of an air blast (6).

In the present work the sedative effect of sodium pentobarbital (Nembutal) was tested on thyro-parathyroidectomized rats.   Thirteen rats which gave positive responses from 1 to 3 days prior to the test were injected with doses of 2 mgm. sodium pentobarbital per 100 grams body weight.   Nine of these gave completely negative results and 4 showed only a fleeting effect which lasted for the duration of the current upon electrical stimulation.   With the sound stimulus, the response was completely negative in all trials.   From 24 to 48 hours after the negative tests the same animals responded positively to both electrical and

sound stimuli. Sedative doses of barbiturates therefore have the same effect against tetany in parathyroid deficiency as they do in magnesium deficiency in the rat.

Spinal transection. In thyro-parathyroidectomized dogs, Noël Paton, Findlay and Watson (5) observed that the tremors and jerkings persisted in muscles below the point of section of the cord but that the sustained tonus was eliminated. The movements of the hind legs were entirely independent of the condition of the fore part of the body. West (4), on the contrary, states that all elements of tetany were retained in the hind limbs when the spinal cord had been transected in the cervical or dorsal region.

The present experiments demonstrated that shortly after spinal transection at about the seventh thoracic vertebra, tetanic seizures were abolished in the hind extremities but the fore part of the body reacted as before operation in magnesium deficient, thyro-parathyroidectomized and citrate injected rats. In magnesium deficient animals, 8 positive reactions were obtained with the fore part of the body while the hind quarters remained limp (after spinal transection). Two of these attacks occurred spontaneously and six after stimulation with the air blast. When the sound was turned on, the rats attempted to run in circles, dragging along the paralyzed hind limbs. Application of the electric current caused tonic extension of the hind limbs for the duration of the current only, up to 2 days after the operation.

Spinal transection was performed on a group of 18 thyro-parathyroidectomized rats maintained on the low calcium diet. All the animals were tested for their susceptibility to tetany prior to the operation. There were 16 positive responses, 10 by galvanic shock and 6 by means of the air blast. Sixteen rats survived the operation and of these, 11 reacted with the fore body while the hind quarters remained limp on being subjected to galvanic stimulation. This response could be elicited in from 2 hours to several days after the spinal transection.

Three citrate injected shock animals following spinal cord transection reacted with typical attacks of tetany in the fore quarters while the hind limbs were affected only for the duration of the current upon being subjected to galvanic stimulus.

Curare. Hartridge and West (3) concluded that sub-paralytic doses of curare abolished the spasms of tetany in thyro-parathyroidectomized dogs. This was offered as proof of the peripheral neuro-muscular nature of tetany. It was previously shown that the tetany of magnesium deficiency is not prevented by the administration of curare. In the present study, the effect of curare was tested on rats subjected to the thyro-parathyroidectomy-low calcium and on the fasting rickets regimens.

In the thyro-parathyroidectomized animals, out of 11 cases in which tetany could be elicited before injection, tetany occurred in 10 curare[5] injected animals when subjected to electrical stimulation. Twenty-five curare injected rats

---

[5] Curare dose from 0.15 to 0.25 mgm. per 100 grams body weight.

were tested in all, employing the different stimuli, with a positive response in 13 cases.

In three fasting rachitic rats which gave a positive reaction prior to injection of curare, 2 suffered convulsions and the third became paralyzed upon electrical stimulation from 10 to 25 minutes after curare administration.

DISCUSSION. The experiments described here demonstrate the essential similarity of the manifestations of tetany produced by the different experimental regimens.[6] The convulsive attacks are indistinguishable from one another. The difference in the effectiveness of the sound and electrical stimuli is not easy to explain. To some degree it may be determined by the severity of the condition since the thyro-parathyroid animals, in which the tetany was very severe, responded strongly to both stimuli.

In many of the conditions in which tetany develops, there is a marked reduction in the serum calcium level, presumably the ionic calcium. Reduction of the serum calcium alone if it is produced slowly, as by low calcium diet, does not lead to tetany in the rat. Perhaps vitamin D and the parathyroid glands produce a reaction which counterbalances the effect of the low serum calcium?

The present experiments confirm the importance, noted in earlier work, of the central nervous system for the manifestations of tetany. The integrity of the central nervous system at a level higher than the spinal cord seems to be essential for sustained tonic convulsions. Evidence that states leading to tetany affect definite parts of the central nervous system is indicated by the work of Gellhorn and Feldman (21). These authors found that the sympathetico-adrenal system becomes sensitized and the vago-insulin system becomes depressed in magnesium deficiency. The recent studies on neurotic states in rats (22) also are of some significance. The sound of the air blast as a stimulus and certain features of the neurotic convulsions are reminiscent of the manifestation of tetany in the rat.

SUMMARY

1. A study has been made of the relation of certain dietary and hormonal factors to the production of tetany in the rat.

Extreme lack in the diet of calcium alone does not produce tetany even though the blood calcium drops to extremely low levels. Tetany will result if the diet is deficient both in vitamin D and calcium. On the low calcium diet, tetany is produced by removal of the parathyroids and even more effectively by removal of the thyroid and parathyroid glands with the addition of adequate thyroid extract to the diet.

2. Rats on different regimens show a difference in response to various stimuli, but the tetanic attacks produced by all the different procedures pursue a like course. Attacks can be induced with a galvanic current but not with the sound of an air blast in tetany associated with vitamin D and calcium deficiency or

---

[6] Since this was written we have demonstrated that in tetany caused by alkalosis in the rat, there is the same kind of convulsion and the same response to spinal transection as described here.

with healing rickets. Both stimuli are effective in tetany due to thyro-parathyroidectomy, and the air blast only is a very effective stimulus in the tetany of magnesium deficiency.

3. Involvement of the central nervous system at a point higher than the spinal cord in the syndrome of tetany is shown by the following: a, sedative doses of amytal and pentobarbital prevent the onset of attacks; b, spinal transected rats reacted with the forebody but not with the hind extremities during attacks, and c, subparalytic doses of curare do not prevent the convulsions of tetany.

REFERENCES

(1) GREENBERG, D. M., M. D. D. BOELTER AND B. W. KNOPF. J. Biol. Chem. **133**: xxxviii, 1940.
(2) DRAGSTEDT, L. R. Physiol. Rev. **7**: 499, 1927.
(3) HARTRIDGE, H. AND R. WEST. Brain **54**: 312, 1931.
(4) WEST, R. Brain **58**: 1, 1935.
(5) NOËL-PATON, D., L. FINDLAY AND A. WATSON. Quart. J. Exper. Physiol. **10**: 233, 1916–17.
(6) GREENBERG, D. M. AND E. V. TUFTS. This Journal **121**: 416, 1938.
(7) BRYAN, W. R. AND W. E. GARREY. This Journal **98**: 194, 1931.
(8) KRUSE, H. D., E. R. ORENT AND E. V. McCOLLUM. J. Biol. Chem. **96**: 519, 1932.
(9) BOELTER, M. D. D. AND D. M. GREENBERG. J. Nutrition **21**: 61, 75, 1941.
(10) TEMPLIN, V. M. AND H. STEENBOCK. J. Biol. Chem. **100**: 209, 1933.
(11) JONES, J. H. AND B. N. E. COHN. J. Nutrition **11**: 293, 1936.
(12) SHOHL, A. T. AND F. C. BING. This Journal **86**: 633, 1928.
(13) HESS, A. F., M. WEINSTOCK, H. R. BENJAMIN AND J. GROSS. J. Biol. Chem. **90**: 737, 1931.
(14) TWEEDY, W. R. AND S. B. CHANDLER. This Journal **88**: 754, 1929.
(15) HOSKINS, M. M. Endocrinology **19**: 453, 1935.
(16) SHELLING, D. H. J. Biol. Chem. **96**: 195, 1932.
(17) HOSKINS, M. M. AND S. B. CHANDLER. Anat. Rec. **30**: 95, 1925.
(18) HOSKINS, M. M. Endocrinology **15**: 324, 1931.
(19) OVERHOLSER, M. D. Anat. Rec. **41**: 303, 1929.
(20) NOËL-PATON, D. AND L. FINDLAY. Quart. J. Exper. Physiol. **10**: 203, 1916–17.
(21) GELLHORN, E. AND J. FELDMAN. This Journal **134**: 603, 1941.
(22) MAIER, N. R. F. Studies of abnormal behavior in the rat. New York, 1939.

A STUDY OF THE CARDIOVASCULAR CHANGES INDUCED BY STIMULATION OF THE MOTOR CORTEX IN DOGS[1]

SIAO-HSI HSU, KAO HWANG AND HO-NIEN CHU

From the Department of Physiology, National Hsiang Ya Medical College, Kweiyang

Recent studies of electrical stimulation of the motor cortex in different mammals have revealed the fact that various autonomic reactions such as pressor and depressor effects, change of heart rate, pupillary dilatation and retraction of nictitating membrane, could be obtained. Dusser de Barenne et al. (1) have observed falls of blood pressure following stimulation of the motor cortex in dogs, cats and rabbits. Hoff and Green (2) obtained chiefly pressor effects in cats and monkeys. Later Green and Hoff (3) studied the limb and renal volume changes in cats and monkeys following cortical stimulation. They reported that either a rise or a fall of blood pressure may result, that renal volume is usually decreased during rise of blood pressure and the limb volume is usually increased regardless of any alteration of the mean blood pressure. The discrepancy of the findings between Dusser de Barenne and Hoff and Green has been attributed to the difference of anesthetics or the character of the cerebral representation of the vasomotor system, as the former used ether, urethane or morphine while the latter used ether or dial ciba supplemented with ether. The purpose of our present study is to determine whether pressor or depressor effects will result from stimulation of the motor cortex in dogs when such animals are under chloralose anesthesia. Also the possible mechanism of any cardio-vascular changes following cortical stimulation will be studied.

METHODS. Twenty successful experiments on dogs are used for this report.

Anesthesia. All animals were anesthetized by intravenous injection of chloralose (70 mgm. per kgm.) with ether induction. In a few experiments, ether alone was used.

Experimental procedure. Blood pressure was recorded from the common carotid by a mercury or membrane manometer. The vasomotor reaction was registered by volume changes of the kidney. Respiratory movements were recorded by means of a trocar inserted through the chest wall or by a tracheal cannula connected with a tambour or water manometer. In a few experiments curare was given in sufficient doses to paralyze muscular movements and artificial respiration was instituted. In some dogs both vagi were sectioned. The skull was exposed either on the left or on the right side by trephining a hole and widening by bone forceps. As soon as the dura was opened, the brain tissue was covered with pledgets soaked in warm Locke's solution.

Electrical stimulation. Bipolar nichrome and platinum wire electrodes separated at the tip less than 1 mm. were used. Each stimulation lasted from 10 to 20 seconds and the interval between two stimuli was two minutes or more in

order to avoid possible extinction and inhibition phenomenon as noticed by Dusser de Barenne and McCulloch (4). Two dry cells of 3 volts connected with a Harvard inductorium were used for stimulation. The strength of current was varied by setting the secondary coil on successive points 1 cm. apart from 2 to 9 cm. In the figures a represents 2 cm., b 3 cm., c 4 cm., etc. Both focal and nonfocal points of the motor area were stimulated as shown in figure 1. Besides blood pressure, respiration and renal volumes were registered, pupillary reaction, variation of heart rate and muscular movements were also observed and recorded during the course of the experiment.

RESULTS. 1. *Depressor effect.* In practically every experiment we obtained a fall of blood pressure upon stimulation of the motor focal points of the sigmoid gyrus in dogs. In a few instances pressor effects were also observed but only
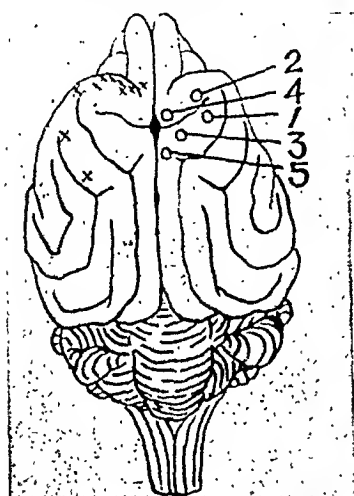
Fig. 1.
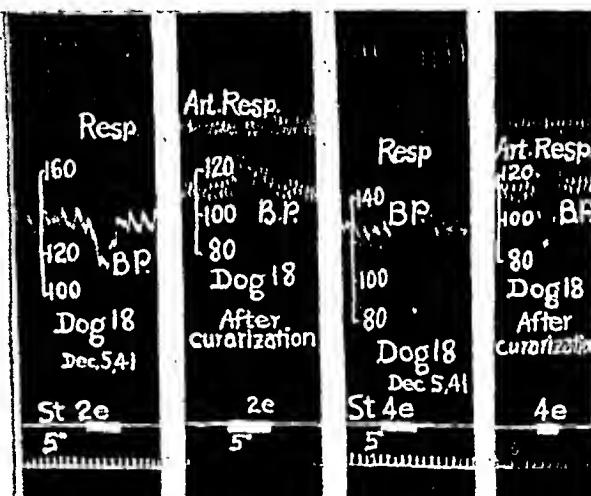
Fig. 2.

Fig. 1. Motor focal and nonfocal points stimulated. o marks focal points and x marks nonfocal points. Focal point 1 represents fore limb area, 2 neck muscle, 3 hind limb, 4 pupil dilatation and 5 tail movement.

Fig. 2. Pressor and depressor effects induced by cortical stimulation after curarization.

at the beginning of stimulation, i.e., an initial rise followed by a fall of blood pressure. In some curarized dogs, however, (fig. 2b) a rise of pressure was observed. In animals injected with curare, stimulation of the motor area may cause either a rise or a fall of blood pressure. This confirms the results of Green and Hoff (3).

2. *Motor focal points.* When liminal current was used to stimulate the sigmoid gyrus only the motor focal points would give rise to blood pressure changes. With a current stronger than liminal, spots other than focal points in the sigmoid gyrus might cause depressor effects; but this, we believe, is due to the spread or irradiation of current to the focal points. The liminal current which would cause depressor effects was usually when the secondary coil of the inductorium was set at 7 or 8 cm.

3. *Variation of depressor effects.* The extent of variation of the depressor

effect is determined by two factors: 1, strength of current, and 2, differences in the focal points. Figure 3*a*, *b*, *c* shows the effects of different strengths of current on the depressor effect. Stimulation of focal point 1 (fore limb area, fig. 1) with current strength f, or 7 cm., resulted in a fall of blood pressure of about 10 mm. Hg, while with current strength d, or 5 cm., the drop of blood pressure could
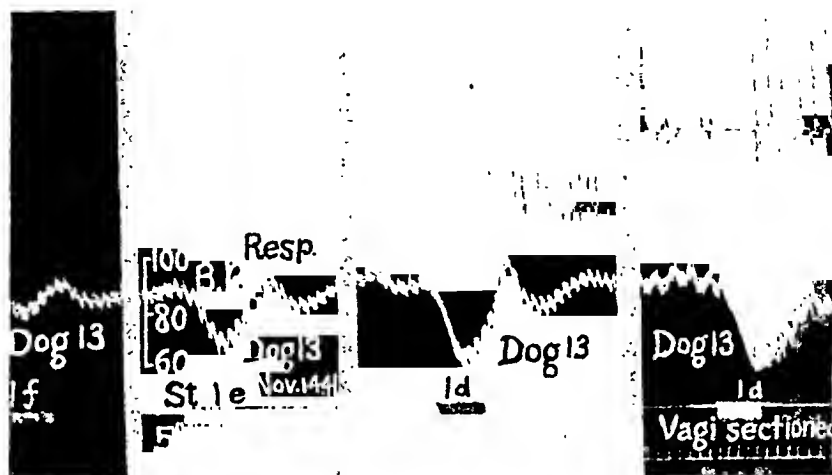
Fig. 3. *a-c* shows the effect of strength of current on the fall of blood pressure. *d* shows that the depressor effect is not influenced by section of the vagi.

a	*b*	*c*	*d*	*e*	*f*	
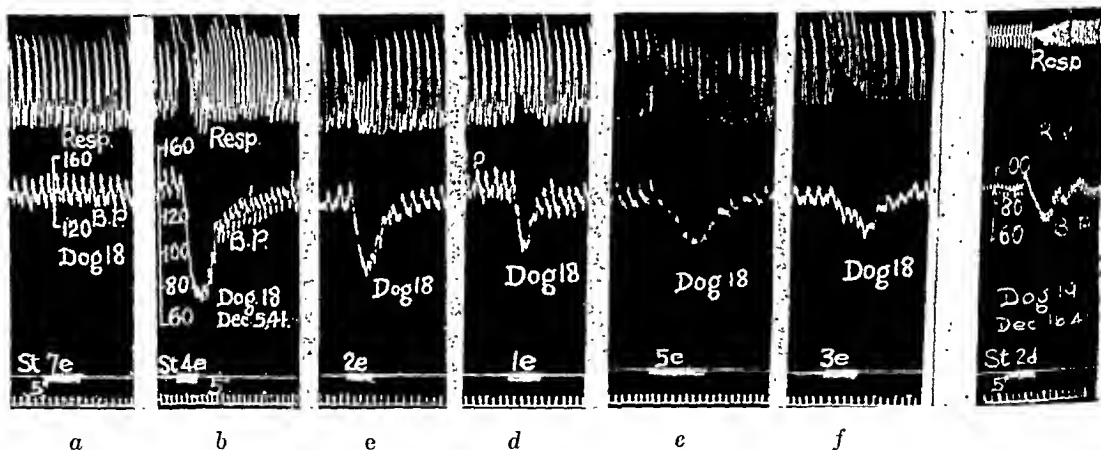
Fig. 4

Fig. 5

Fig. 4. *a* shows the effect of stimulation of one of the non-focal points; *b–f* shows the variation of depressor effect by stimulation of different focal points of the motor cortex.

Fig. 5. The depressor effect accompanying the increase of renal volume following cortical stimulation.

be over 40 mm. Hg. The drop of blood pressure from stimulation of any focal point was not influenced or abolished by section of both vagi (fig. 3*d*). With the same strength of current, different focal points, however, did not give the same effect. Focal points such as pupillary dilatation, neck and fore limb area usually gave more marked depressor effect than those of tail and hind limb areas (fig. 4 *a–f*).

4. *Relationship between the depressor effect and muscular movements.* When the strength of current was sufficient to induce a motor response conspicuous depressor effects and renal volume changes usually resulted. When the strength of current was below the threshold for muscular movement noticeable change of blood pressure did not occur except when focal point 4 (pupillary dilatation center) of the sigmoid gyrus was stimulated. However, in view of the observations that in curarized animals marked depressor effects were obtained from all the motor focal points without the slightest accompanying muscular movements, and that in a few instances there were muscular movements without accompanying depressor effects, we feel that the depressor effect itself is independent of muscular movements.

5. *Heart rate.* The heart rate is usually increased on cortical stimulation. In four experiments using a membrane manometer to register blood pressure, it was found that before stimulation the average heart rate was 110 per minute; during and shortly after stimulation it had increased to 132 per minute, with an average increase of approximately 20 per cent. The increase in heart rate usually began after ten seconds of stimulation, similar to the duration of the latency of the depressor effect. It is evident that the change of blood pressure was not dependent on the change of heart rate since these changes occurred simultaneously and since depressor effect was obtainable after section of both vagi.

6. *Renal volume.* On stimulation of the motor focal points in dogs there was always an increase of renal volume accompanying the fall of blood pressure (fig. 5). Green and Hoff (3) observed "diminution of renal volume in curarized animals under ether anesthesia during the rise of blood pressure evoked by stimulation of the motor cortex" in cats and monkeys. Their result is contrary to our present findings in dogs anesthetized with chloralose. Whether the disagreement of our findings with that of Green and Hoff (3) is due to the difference of anesthetics or to cortical representation needs further experimental clarification. There is very little doubt that the depressor effect resulting from cortical stimulation is chiefly due to the vasodilatation of the visceral organs.

7. *Respiratory movements.* In most cases when the motor focal points were stimulated the amplitude of the respiratory movements was decreased and the rate increased. In a few cases apnea resulted as long as the stimulation lasted (figs. 2–5). The findings on the variation of the respiratory movements in relation to the fall of blood pressure confirm the results of Chu and Loo (5) in their experiments on hypothalamic stimulation in cats. The change of blood pressure in our experiments, however, was not due to the change of the respiratory movement as both pressor and depressor effects were still obtainable after paralysis of the respiratory muscles by curarization (fig. 2).

DISCUSSION. Recent experiments on cortical stimulation and ablation of the motor areas (including motor and premotor areas) have shown that the motor area of many mammals serves not only as the center for voluntary movement but also represents the cortical autonomic center. That the activity of the gastrointestinal tract can be augmented in the presence of ablation of the premotor area has been demonstrated by Sheehan (6) and Watts and Fulton (7). Watts has also shown that augmented activity of the gastrointestinal tract can be induced

by stimulation of this region (8). From the study of vasomotor changes by cortical stimulation, Green and Hoff (3) concluded that "there are certain nervous pathways, originating in the motor cortex, excitation of which results in a redistribution of blood."

Our present findings on cardiovascular and other autonomic changes further strengthen our concept of cortical autonomic representation in the motor area. Our results, that the intensity of the depressor effect is dependent on the strength of current and on the seat of motor focal points—the fore limb area is more sensitive and effective than the hind limb area—lead us to believe that there are two possibilities for this mechanism. 1. Probably from the motor area there are thinly myelinated or unmyelinated fibers mingled with the cortical-spinal or the extrapyramidal system which make connections with various autonomic centers such as cardio-inhibitory, respiratory and vasomotor centers in the hypothalamic, tegmental and bulbar regions. 2. Through direct or indirect pathways, connections between cortico-spinal and extrapyramidal system fibers and the autonomic centers in the lower levels are made.   The existence of these hypothetical tracts certainly needs to be verified by experimental studies with the Marchi method and careful examination of silver preparations by neuroanatomists.

SUMMARY

1. By stimulation of the motor focal points on the sigmoid gyrus of the dog anesthetized with chloralose, there is always a fall of blood pressure. The depressor effect thus produced is always associated with the muscular movement in noncurarized animals, but the production of the former is not dependent on the latter.

2. The intensity of the depressor effect evoked from the cortical stimulation in dogs is determined by the strength of the stimulating current and by the particular motor focal point that is stimulated.

3. The heart rate is usually increased about 20 per cent on stimulation of the motor area in dogs, but the change of the heart rate is not a causal factor in the depressor effect, since the latter is not influenced or abolished by section of both vagi.

4. The respiratory movements are usually decreased in amplitude and increased in number, and occasionally apnea may occur during cortical stimulation.

5. The renal volume is always increased accompanying the fall of blood pressure following cortical stimulation.   Vasodilatation of the visceral organs is probably the cause of the depressor effect.

REFERENCES

(1) DUSSER DE BARENNE, J. G. AND F. KLEINKNECHT. Ztschr. f. Biol. 82: 13, 1924.
(2) HOFF, E. C. AND H. D. GREEN. This Journal 117: 411, 1936.
(3) GREEN, H. D. AND E. C. HOFF. This Journal 118: 641, 1937.
(4) DUSSER DE BARENNE, J. G. AND W. S. McCULLOCH. This Journal 118: 510, 1937.
(5) CHU, H. N. AND Y. T. LOO. Chinese J. Physiol. 11: 295, 1937.
(6) SHEEHAN, D. J. Physiol. 83: 177, 1935.
(7) WATTS, J. W. AND J. F. FULTON. New England J. Med. 210: 883, 1934.
(8) WATTS, J. W. J. A. M. A. 104: 55, 1935.

THE BLOOD PRESSURE OF THE FETAL RAT AND ITS RESPONSE TO RENIN AND ANGIOTONIN[1]

PAUL BURLINGAME, J. A. LONG AND ERIC OGDEN

*From the Department of Zoology and Division of Physiology of the Medical School,
University of California*

Knowledge of the blood pressure norms for the whole of the postnatal period of life, particularly in man, is extensive but a comparable body of information relating to the prenatal period has not been forthcoming presumably because less readily available rather than less interesting. The circulatory system before birth is not subject to reflex disturbances to the same extent as the adult but it is itself undergoing very considerable structural changes.

From time to time single observations upon fetal blood pressure have appeared (reviewed by Barcroft, 1936) but only one study has been made of fetal blood pressure in relation to fetal age, the seven measurements of the sheep fœtus by Barcroft. For a clear understanding of the changes in blood pressure during the course of intrauterine life it is necessary to have a large number of observations at short intervals throughout gestation in some one form of animal from which a continuous supply of embryonic material of known age can be obtained. The rat was chosen because of a need of some knowledge of blood pressure in certain experimental work projected by two of us, and because of the possibility of correlating pressure changes with the known morphological steps in the development of the heart in the rat (Burlingame and Long, 1939). A disadvantage lies in the relative immaturity of the rat at birth, as compared with sheep, dog and cat, animals from which isolated blood pressure measurements have been obtained near the end of the gestation period.

METHODS. *Preparation of the mother rat.* The time of conception was arbitrarily dated from the midnight preceding the finding of sperm in the vagina; and was confirmed by reference to the morphological data obtained by Long and Burlingame (1938). The pregnant rat was lightly anesthetized with ether by inhalation followed with 0.5 mgm. of pentobarbital sodium by intravenous injection repeated at half-hour or longer intervals.

Since in many instances the maternal blood pressure was recorded simul-

taneously with the fetal blood pressure, the carotid artery was exposed. The trachea was cannulated to minimize the respiratory difficulties incident to the prolonged anesthesia. After a 3 inch abdominal incision was made in the midline, and any consequent bleeding controlled, the mother was immersed in a bath of Tyrode's solution kept at 38 ± 0.5°C. One horn of the uterus was lifted out and gently extended on a submerged cork platform. Through a short incision at the cephalic end of the uterus a slender, slightly curved, glass rod was inserted a short distance between the wall of the uterus and the yolk sac, and used both to elevate the uterus momentarily above the surface of the water and as a guide (protection to the fetal membranes) in opening the uterus. The uterus was opened by making an incision throughout its length by means of a hemostatic cautery along the rod as the latter was inserted further and further beneath the wall. The uterus was then pinned out on the cork platform in such a manner as to bring the placenta of one selected fetus into a horizontal position. Care was taken not to separate any of the placentae from the uterus since this accident causes profuse bleeding which lowers the maternal blood pressure. Any unnecessary manipulation which might result in obstruction to the uterine circulation was carefully avoided.

The maternal blood pressure from the carotid artery was recorded on a smoked drum by means of a Hurthle manometer. Ten per cent citrate was used as an anticoagulant.

Preparation of the fetus. All subsequent operations were performed under the dissecting microscope which was supported on a jointed bracket for flexibility of positions. The yolk sac of the chosen fetus was opened with the cautery, the incision being made between parallel vessels to avoid bleeding and to cause as little interference with the yolk sac circulation as possible. The yolk sac was then slipped off the embryo, revealing the umbilical vessels and their branches on the surface of the placenta. Care was taken not to stretch or manipulate the umbilical vessels because of their tendency to contract when disturbed. A minute incision was made with sharp needles in the tough transparent membrane which covers the surface of the placenta, above a suitable branch and not too close to the umbilical artery. The branch was then scraped free of connective tissue which might clog the cannula.

The cannula, about 7 mm. long, was attached to a short length of catheter tubing which was supported by a light clamp on racks and pinions about 2 cm. from the tip. Its position could be adjusted in both the vertical and horizontal planes to any convenient angle (usually at nearly right angles to the long axis of the uterus). The length of tubing between the cannula and the support allowed sufficient flexibility to prevent the blood vessel from pulling away from the cannula during uterine contractions which occur during and after the eighteenth day of pregnancy. This arrangement permitted the cannula to travel with the placenta without disturbing the connection. In each instance a cannula was chosen which would fit the blood vessel snugly. A tiny barb, on the fish hook principle, was drawn on the tip as an additional precaution against the pulling out of the cannula. The tip, 0.15 to 0.25 mm. in diameter, was

bevelled like a hypodermic needle and fire polished. The cannula was brought into position parallel to and just above the prepared vessel with its tip close to the junction of the branch and the umbilical artery. It was possible, by grasping the edges of the incision above the blood vessel to pull the vessel onto the cannula without loss of blood inasmuch as the tip was sharp enough to pierce the blood vessel wall. The fetal circulation through the placenta was not obstructed except in the one branch. Heparin was used as an anticoagulant and was drawn into the cannula just before its insertion.

The method used in determining the fetal blood pressure was essentially that devised by Landis (1934) for determining the blood pressure in human capillaries. The fetal blood pressure was read from a capillary water column (Tyrode solution) which was balanced with the blood pressure by means of a syringe; that is to say, the water column was continuously adjusted to a point at which blood just remained in the cannula but no Tyrode entered the artery during diastole. The rise in the tube due to capillarity and the exact level of the fetus were taken into account in setting the zero point. Readings were taken at intervals of 15, 30 or 60 seconds during periods of fluctuations and at longer intervals when a particular level was maintained. Under these conditions the fetal pulse rate maintained a constant level for an hour or more.

The above procedure applies to embryos of $15\frac{1}{2}$ days or more. The method was not found feasible for embryos in the earlier stages of pregnancy because of the tendency of the uterus to expel the embryo through an incision.

Moreover, it soon became apparent that when the younger embryos are separated from the uterus and placed in Tyrode solution at body temperature the pulse rate remains normal for only a few minutes. If the embryo with membranes intact is placed in a bath at room temperature, the pulse rate falls immediately to a low level but the rate quickly returns to normal if the embryo is subsequently transferred to a bath at body temperature. The normal rate is then maintained as long as though the embryo had been placed in a warm bath immediately upon removal from the uterus. These early embryos were therefore placed in a room temperature bath for the operative procedure preparatory to cannulation in order that the entire period of normal pulse rate might be used for the blood pressure determination.

The yolk sac was severed from the placenta by cutting with iridectomy scissors in the narrow non-vascular zone adjacent to the placenta, thus allowing the yolk sac to slip down the umbilical cord exposing the branches of the umbilical vessels on the inner surface of the placenta. There was no loss of fetal blood inasmuch as the yolk sac vessels remained entirely intact. . (Occasionally an anastomosis was encountered between the yolk sac and the placental circulation, across the normally nonvascular zone, in which case the embryo could not be used.) A branch of the umbilical artery was then prepared for cannulation, as described above, and the embryo transferred to the warm bath. The usual time interval between removal from the uterus and cannulation was from five to ten minutes.

Although no precise data were collected on the subject it was found that

embryos could be kept at room temperature for a half hour or more without impairing the ability of the heart to return to its normal rate upon restoration of the normal temperature.

Both methods were employed with $15\frac{1}{2}$ day embryos for comparison.

Records were made of the crown-rump length of each fetus (measured wet). The length of each fetus actually used did not in any case differ by more than one millimeter from the average for the litter. The average crown-rump length of the fetuses from which blood pressure measurements were obtained is given at the foot of figure 2 for each age group. More extensive data have been compiled by Gonzalez (1932) on the rate of growth of the rat fetus. Our measurements in the latter half of pregnancy are somewhat in excess of his mean values but fall within the range of normal variation which he reports.

Standard conditions. The following criteria were used to insure as far as possible that the fetuses were in a suitable physiological condition for observation. 1, no maternal hemorrhage; 2, no obstruction in the uterine blood supply as a result of kinking or stretching; 3, characteristic arterial color in the umbilical artery; 4, uninterrupted, constant and rapid pulse in the umbilical artery; 5, no loss of fetal blood.

OBSERVATIONS. *The pulse rate.* A rapid and steady pulse rate was taken as the primary criterion for normalcy. The pulse was therefore observed and recorded at frequent intervals for each fetus (fig. 1). Two curves (joined by a dotted line) appear in this figure. The upper curve shows the increment in pulse rate from $15\frac{1}{2}$ to $21\frac{1}{2}$ days for embryos with placental attachment to the mother. The lower curve shows the observations between the ages of $11\frac{1}{2}$ and $15\frac{1}{2}$ days for embryos entirely separated from the mother. Each point in the curves represents an average of the several observations on different embryos at any particular age.

Since the points for $11\frac{1}{2}$, $12\frac{1}{2}$ and $13\frac{1}{2}$ days are in line with the points of the upper curve, these three pulse rate values for embryos without placental attachment were considered to be within the range of normalcy. The blood pressure values for these three age groups were therefore considered acceptable and are plotted in figure 2. Inasmuch as the average pulse rate value for embryos of $15\frac{1}{2}$ days without placental attachment (lower curve) is considerably lower than the average rate for embryos for the same age with placental attachment the lower value was considered abnormally low and the blood pressure values for these embryos were therefore discarded. The blood pressure values for $14\frac{1}{2}$ day embryos were discarded for the same reason.

It is apparent from these two curves that the pulse rate in the very early stages of pregnancy is independent of placental attachment to the uterus, for a brief period. After $13\frac{1}{2}$ days, embryos cannot be depended upon to maintain a normal pulse rate long enough to give a normal blood pressure reading after separation of the placenta from the uterus.

Goss (1938) fixes the time of first contraction of cardiac muscle cells at 9 days 14 ± 2 hours (dating from "the early hours" of copulation) and the starting rate 37 to 42 per minute. The embryos were observed in hanging drops at

38°C. Goss' value is placed on our graph for comparison with our data (fig. 1). Goss does not comment on the curious fact that he obtained no value less than 37 beats per minute.

The only other values for the pulse rate of the rat fetus are those of Corey (1932) which are considerably lower than ours.

Blood pressure changes. The blood pressure records of each fetus were plotted against time in minutes. Typical examples are shown in figure 3. Because the differences in pressure are small, all values for fetal blood pressure are expressed in terms of millimeters of Tyrode solution rather than of mercury.
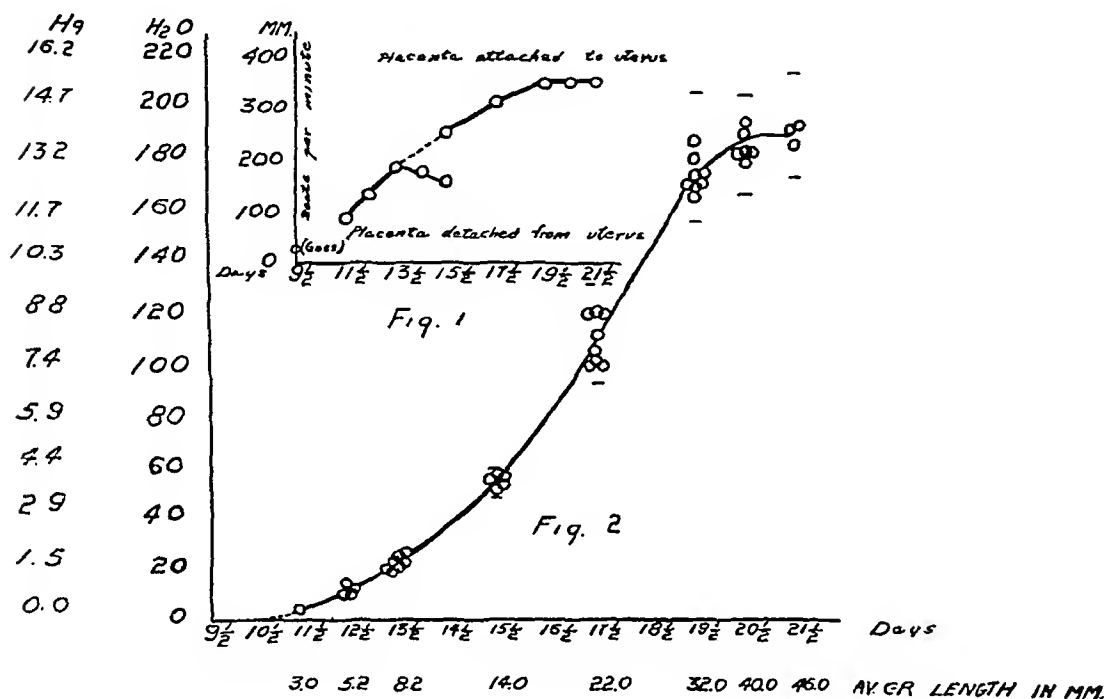
Fig. 1. Change in heart rate of fetal rats with time of gestation. Each circle represents the mean value for the age group.

Fig. 2. Change in blood pressure of fetal rats with time of gestation. Each circle represents a value for one fetus (see text). Bars show extreme values for single observations in each age group.

The $15\frac{1}{2}$ day fetus in figure 3, maintained a value which fluctuated only ±1.0 mm., from 64 to 68 mm. (5 mm. Hg) over a period of 47 minutes (26 min. of which are shown). The average fluctuation for the group was 0.9 mm. Tyrode.

At $17\frac{1}{2}$ days, in the example shown in figure 3, there is a fluctuation of ±7.0 mm., from 104 to 120 mm. (8 to 9 mm. Hg). The average fluctuation of five fetuses from which readings were obtained at frequent intervals for at least twenty minutes was ±6.0 mm. The records for the other three members of the group were not of sufficient duration or the readings too infrequent to show fluctuations which may have occurred.

The record of the $19\frac{1}{2}$ day fetus in figure 3 shows a fluctuation of 15.0 mm.

from 164 to 194 mm. (12.1 to 14.3 mm. Hg).   The average fluctuation of three fetuses from which records were obtained at frequent intervals for twenty minutes or more was ±14.0 mm.

The greatest fluctuation was shown by $21\frac{1}{2}$ day fetus with a range of 23 mm. from 168 to 214 mm. (12.4 to 15.7 mm. Hg).

The selection of any representative value for the blood pressure after the sixteenth day was an arbitrary matter because of the prolonged period of fluctuation in pressure.   It was necessary to choose such a value however in order to study the relationship between pressure and age.   The procedure used was as follows:

1. Data obtained after the 30th minute were discarded as representing possibly a moribund state.

2. In those fetuses which showed fluctuations in blood pressure, an average was taken of the highest and lowest points.   Thus in figure 3, $19\frac{1}{2}$ days, the
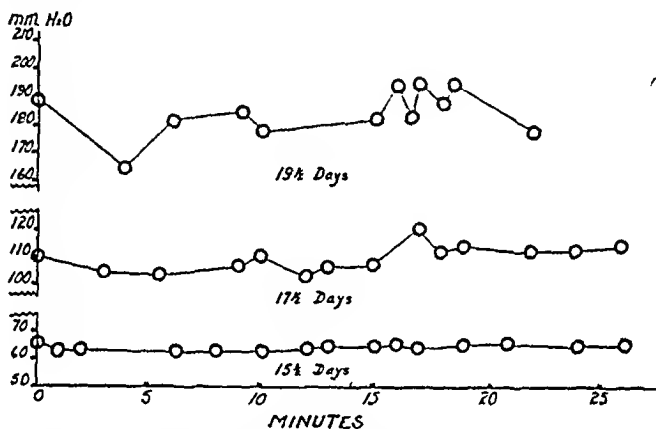
Fig. 3. Blood pressure variations during period of observation.   Each line gives observations on one fetus selected to represent its age group.

blood pressure is read 179 ± 15 mm. Tyrode (13.2 mm. Hg), the lowest value being 164 mm. and the highest 194 mm.

3. Several fetuses showed a steady gradual rise in blood pressure during the first few minutes and then maintained a level, with or without fluctuations, sometimes considerably above the initial pressure.   In such cases the first few minutes were discarded as representing possibly a period of recovery from manipulation.

4. In the case of embryos of $15\frac{1}{2}$ days and younger which were separated from the uterus the first indication of a level having been reached after the bath temperature was raised from 24°C to 37.5°C was taken as the blood pressure of the embryo.   Since embryos of $15\frac{1}{2}$ days show fluctuations of only 1.0 mm., it was assumed that fluctuations of greater magnitude do not occur in earlier periods of gestation.

The mean pressure was computed for each fetus and for each age group. These values are plotted in figure 2 to show the progressive rise in fetal blood pressure with age.   The highest and lowest values recorded within each age

group are shown by bars. In studying this curve it should be recalled that there is the possibility of a timing error of six hours or so in the age of the fetus.

Maternal blood pressure. Determinations of the maternal blood pressure were made in order to be sure that the maternal pressure was being maintained within normal limits under the conditions of the experiment. The average of twenty observations was 129.35 mm. Hg (standard deviation of the distribution 23.82). Byrom and Wilson (1938) have reviewed the work on the blood pressure of the adult rat, the pressure range being from 78 to 154 mm. Hg. There was no evident parallelism between fluctuation in maternal and fetal pressures in our experiments.

DISCUSSION. Goss (1938) fixes the time for the beginning of circulation at "approximately 12 hours beyond the stage of first contraction" which would be very early in the 10th day; Burlingame and Long (1939) at about 10½ days. The blood pressure curve, if extended downward, would meet the base line (zero pressure) at a point quite in agreement with these findings (dotted line, fig. 2).

Between the 21st and 22nd day (the normal time for parturition) the curve levels off rather abruptly. It is felt that perhaps little significance should be attached to this deflection, inasmuch as the average for the 22nd day represents only three values. We found difficulty in maintaining the normal pulse rate of a 21½ day fetus in the saline bath for more than a few minutes. Moreover the attachment of the placenta to the uterus at this age is so extremely tenuous that the least disturbance results in complete detachment or serious hemorrhage. However one fetus was maintained under satisfactory conditions for 28 minutes and showed at the end of this period the highest pressure recorded for any age, 214.0 mm. (15.7 mm. Hg). It is likely that the average given for 21½ days, 189 mm. (13.9 mm. Hg), is from 5 to 10 mm. (less than 1.0 mm. Hg) too low. The only other available estimate of the fetal rat's blood pressure is that of Corey (1932) who gives an average value of 10 mm. Hg based upon observation on three litters during the latter half of pregnancy.

The blood pressure of the fetus at birth (about 14.0 mm. Hg) is considerably lower than that of other animals which have been investigated. The blood pressure of other animals near term ranges from 30 to 80 mm. Hg according to Barcroft (1936). Perhaps this is due to the immaturity of the rat at birth as compared with the cat, dog, guinea pig or sheep. If the rate of increase shown by the rat fetus between the 20th and 21st day, 1.3 mm. Hg per day (the 22nd day disregarded for reasons mentioned above) were continued after birth the adult blood pressure would be attained in a little less than three months. There is, however, no available information on the post-natal rate of increase in blood pressure in the rat.

Our own observations and those of other workers suggest that the fluctuations in fetal blood pressure recorded during the latter half of pregnancy are produced by alternate contractions and relaxations of the uterus.

Clark (1932) found that adrenaline, pitressin and histamine injected into the maternal blood stream of a dog or cat resulted in a trifling rise in fetal

blood pressure followed by a prolonged fall. He attributed the decline in pressure to occlusion of arterioles of the placenta during uterine contraction.

Likewise in experiments to be described below we found that the injection into the maternal blood stream of pressor substances (fig. 4) known to cause uterine contraction were followed by sudden and profound falls in fetal blood pressure. These substances when injected directly into the fetal blood stream cause a marked rise in fetal blood pressure.

Structural changes in the heart and blood vessels do not produce any abrupt changes in the functional development of the circulatory system as reflected in the rate of increase of blood pressure and pulse rate. With the transition from a one to a two chambered ventricle during the sixteenth day there is an acceleration in the rate of increase in the blood pressure (fig. 2) and the new rate is maintained without change through the 20th day (straight segment of the curve). With the partitioning of the ventricle into right and left chambers the heart becomes functionally a four-chambered organ although the foramen ovale is still patent and aids in the filling of the left atrium. However, the augmented rate of increase in blood pressure cannot be attributed to any one structural change, radical as it may appear.

Injections of Renin into the Pregnant Rat and Fetus.[2] An examination of the changes in blood pressure of mother and fetus when either of these organisms is exposed to renin is of interest for several reasons. In the first place such a study should throw light on the transmission across the placenta of this substance or its pressor derivatives. Irvine Page and O. M. Helmer (1940) have published evidence that the pressor action of renin is due to the liberation of angiotonin. Angiotonin, which is a strong pressor, is crystalloid and diffusible. It might, therefore, be expected to diffuse across the placenta and accumulate in sufficient concentration to show its pressor action. In the second place since many believe renin to play a part in human hypertension it would be of interest to add to the information accumulated from the few cases in which blood pressure measurements have been recorded from new born infants of hypertensive mothers by F. J. Browne and G. H. Dodds (1936). Further, it has been postulated by Harrison, Grollman and Williams (1940) and denied by Page, Patton and Ogden (1941) that the fetal kidney is instrumental in lowering hypertension in pregnant animals due to the production of an antipressor substance. If this were a specific response of the fetus to maternal hypertension it would seem that the pressor substance or a specific derivative of it must be getting into the fetal circulation.

Method. Renin was injected into the jugular vein in the case of the mother. Injection into the fetal circulation was accomplished by piercing the catheter tubing with a no. 27 hypodermic needle just behind the cannula and then forcing the substance into the umbilical artery with the syringe in the manometer system. Corresponding amounts of Tyrode solution injected into the fetal circulation did not affect the blood pressure. Only fetuses of $17\frac{1}{2}$, $19\frac{1}{2}$ and $20\frac{1}{2}$ days were used in the following experiments.

Normal pressures were recorded from mother and fetus for a period of from ten to twenty minutes prior to the first injection of renin. Chance extraneous stimuli (e.g., manipulation of the uterus) caused sudden falls of maternal blood pressure from which there was gradual spontaneous recovery; but critical examination showed that rises of blood pressure of 5.0 mm. Hg or more above the initial normal level occurred only after the injection of a known pressor substance.

Results. Eight pregnant rats were given one or more injections of renin. The doses used ranged from 0.005 to 0.15 cc. With so few experiments and with the uncertainty as to whether the progress of pregnancy modified the responses to renin it was not possible to relate dosage to effect but the impression was gained that the smaller doses were about as effective as larger ones so far as the effect on blood pressure was concerned.

In every case the first injection of renin into the maternal circulation resulted in a rise in maternal blood pressure averaging 20.6 mm. Hg. The pressor
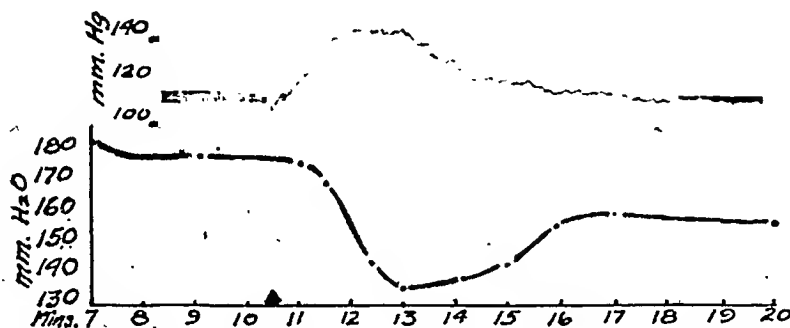
Fig. 4. Effect on maternal and fetal blood pressure of injection of 0.15 cc. renin into maternal blood stream.

response of the mother characteristically reached a maximum after 90 seconds and had largely disappeared within ten minutes.

In no instance was there a significant rise in fetal blood pressure following the injection of renin into the maternal blood stream. In only one was there any rise at all and this increase was not greater than a normal fluctuation. In all other instances the fetal pressure continued to maintain its previous level or showed a sudden or gradual decrease, depending upon the dosage.

An abrupt fall of 42 mm. Tyrode (3.1 mm. Hg) in fetal blood pressure was produced by an unusually large dose (0.15 cc.) to the mother (fig. 4). Doses of renin up to 0.04 or less did not produce this effect.

One rat, in the 21st day of pregnancy, was given a series of injections of renin over a period of forty minutes in doses of 0.01 or 0.02 cc. for a total of 0.11 cc. During this time the maternal blood pressure fluctuated between 140 and 160 mm. Hg (initial level 130 mm. Hg). The fetal blood pressure fluctuated between plus 1.0 and minus 17 mm. in respect to the initial level of 185 mm. Tyrode (13.6 mm. Hg). During the greater part of the period the fetal blood pressure was not more than 5.0 mm. below the initial level. At the end of the

period the fetus was given an injection of 0.06 cc. of renin which produced a rise of 115 mm. (8.5 mm. Hg) above the initial level. The maximum pressure of 300 mm. Tyrode (22.1 mm. Hg) was reached five minutes after the injections. The pressure then gradually fell, returning to the initial level one hour and thirty-eight minutes after the injection. The maternal blood pressure showed no response to the injection of renin into the fetus. Forty-five minutes after the injection of renin into the fetus an injection of 0.05 cc. into the maternal circulation produced a rise of 25.0 mm. Hg in the maternal blood pressure but produced no interruption in the steady decline in fetal blood pressure. Both maternal and fetal blood pressures were recorded for three hours and fifteen minutes in this experiment. Thus the injection of renin into mother and fetus seems to produce entirely independent results confined to the organism receiving the substance and independent of whether the other organism has or has not been recently loaded with renin (tachyphylactic state).

Six fetuses $19\frac{1}{2}$ and $20\frac{1}{2}$ days old were given initial injections of 0.06, 0.06, 0.06, 0.03, 0.03, 0.015 cc. renin. The resultant rises in fetal blood pressure were, respectively, 127, 125, 86, 89, 96, 49 mm. Tyrode (9.3, 9.1, 6.3, 6.5, 7.1, 3.6 mm. Hg). In a seventh fetus, $17\frac{1}{2}$ days old, the first and second doses of 0.01 and 0.02 cc. produced no response but a third injection of 0.02 cc. gave a rise of 31.0 mm. This is in contrast to one $19\frac{1}{2}$ day fetus that received five injections (0.015, 0.015, 0.01, 0.03, 0.02 cc.) in fifteen minutes and failed to respond to any but the first (rise of 49 mm. Tyrode).

It is to be noted that the absolute effective dose per fetus is about the same as the absolute effective dose per mother, indicating that the fetus is vastly less sensitive to this substance than the mother. Furthermore the youngest fetus gave the least response.

Of the thirteen injections given to these seven fetuses only seven were followed by changes in maternal blood pressure and these were inconstant in direction and negligible in magnitude (−2, −3, +4, +4, −2, −2 mm. Hg). Four of the mothers were subsequently demonstrated to be responsive to renin injected into their own circulations and the other two had been previously so tested.

An injection of 0.03 cc. of angiotonin into the blood stream of a pregnant rat produced a rise of 35 mm. Hg. During the subsequent four minutes the blood pressure of the fetus ($20\frac{1}{2}$ days old) fell 72 mm. Tyrode (5.3 mm. Hg) from 197 to 125 mm.

In another experiment 1.0 cc. of Tyrode solution containing 0.08 cc. of angiotonin was perfused into the umbilical artery of a $19\frac{1}{2}$ day fetus over a period of twenty minutes. The maternal blood pressure remained constant at 120 mm. Hg. Subsequently injection of 0.01 cc. of angiotonin into the maternal circulation gave a rise of 30 mm. Hg.

Fetal injections of epinephrine. A pregnant rat with an initial blood pressure of 120 mm. Hg was given an injection of 0.02 cc. (1:10,000) of epinephrine when the pressure had fallen to 66 mm. Hg, after forty minutes. The maternal pressure rose immediately to 102 mm. Hg and the fetal pressure which had

maintained a level of 175 ± 5 mm. Tyrode (12.9 mm. Hg) for fifty minutes, fell to 150 mm. Tyrode (11.0 mm. Hg). In four minutes the fetal pressure returned to 176 mm. Tyrode (12.9 mm. Hg) and in six minutes to 180 mm. Tyrode (13.2 mm. Hg).

In another experiment a $19\frac{1}{2}$ day fetus responded to 0.02 cc. (1:10,000) epinephrine with a rise in pressure of 30 mm. Tyrode (2.2 mm. Hg). This fetus, whose normal blood pressure level was 172.0 mm. Tyrode (12.6 mm. Hg) had shown tachyphylaxis to renin thirty-five minutes previously and at the time of the epinephrine injection was maintaining a level of 100.0 mm. Tyrode (7.4 mm. Hg) following an injection of 0.02 cc. renin to the mother.

The results obtained in these experiments with renin, angiotonin and epinephrine substantiate those of Clark (1932) with pitressin and epinephrine. We did not observe the small transitory rise in pressure which preceded the pronounced fall that is shown in his records probably because of the fact that we did not obtain continuous records of the fetal blood pressure.

CONCLUSIONS

1. Injection of an effective dose of renin into the blood stream of rats in late pregnancy does not affect the fetal blood pressure. Injection of even larger doses of renin, angiotonin and adrenaline into the maternal bloodstream cause a profound fall in fetal blood pressure. Recovery is slow and incomplete.

2. Injections of renin, angiotonin and adrenaline into the fetal blood stream cause a pronounced rise in fetal blood pressure.

3. With renin, tachyphylaxis was demonstrated in both mother and fetus independently but was not transferred from one to the other.

4. Injections of renin and angiotonin large enough to raise the maternal blood pressure when injected directly into the maternal circulation, fail to do so if injected into the fetal circulation.

5. The fetus is very much less responsive to renin, angiotonin and epinephrine than the mother.

Discussion. It seems likely that renin is not a small enough particle to pass across the placenta. This might be expected from what is known of its molecular size. If, however, renin activity is dependent upon the liberation of angiotonin we must conclude that angiotonin also fails to pass the placenta, or if it passes at all that there is enough delay in its passage to prevent its accumulating in effective concentrations on the other side of the placenta. This is further supported by the experiments in which angiotonin was injected.

These concepts must be kept in mind when attempting to attribute the alleged (Harrison, Grollman and Williams, 1940) antihypertensive effect of pregnancy to the activity of the fetal kidney. For it is difficult to postulate a mechanism whereby the fetal kidney could take part in a compensatory adaptation to a substance whose effects do not appear to reach it. This work offers some support to the suggestion of Patton, Page and Ogden (1941) that the antihypertensive effect of pregnancy in the rat is dependent rather upon the maternal changes of pregnancy than upon the fetus.

REFERENCES

(1) ANGULO Y GONZALEZ, A. W.   Anat. Rec. **52**: 117, 1932.

(2) BARCROFT, J.   Physiol. Rev. **16**: 103, 1936.

(3) BROWNE, F. J. AND G. H. DODDS.   Lancet **230**: 1059, 1936.

(4) BURLINGAME, P. L. AND J. A. LONG.   Univ. of Calif. Publ. Zool. **43**: 249, 1939.

(5) BYROM, F. B. AND C. WILSON.   J. Physiol. **93**: 301, 1938.

(6) CLARK, G. A.   J. Physiol. **74**: 391, 1932.

(7) COREY, E. L.   This Journal **101**: 304, 1932.

(8) GOSS, C. M.   Anat. Rec. **70**: 505, 1938.

(9) HARRISON, T. R., A. GROLLMAN AND J. R. WILLIAMS, JR.   This Journal **128**: 716, 1940.

(10) LANDIS, E. M.   Physiol. Rev. **14**: 404, 1934.

(11) LONG, J. A. AND P. L. BURLINGAME.   Univ. of Calif. Publ. Zool. **43**: 143, 1938.

(12) PAGE, E. W., H. S. PATTON AND E. OGDEN.   Am. J. Obstet. and Gynec. **41**: 53, 1941.

(13) PAGE, I. H. AND O. M. HELMER.   J. Exper. Med. **71**: 29, 1940.

THE EFFECT OF PULMONARY VENTILATION ON THE PRESSOR ACTION OF ADRENALINE[1]

GEORGE W. STAVRAKY

From the Department of Physiology, University of Western Ontario, Faculty of Medicine, London, Canada

There are some indications in the literature that changes in pulmonary ventilation may modify the action of adrenaline. For instance, it has been established that the hyperglycemic effect of adrenaline may be decreased, or even reversed, by hyperventilation. The effect of adrenaline on blood pressure, under varied conditions of respiration, has received much less notice. Balint and Goldschmidt (1) have shown that in children hyperventilation greatly reduces the rise of blood pressure caused by the intravenous injection of adrenaline. This observation was, in the main, confirmed by Brehme and Popovicin (2). Duzár and Fritz (3), however, who investigated the effect of hyperventilation in cats, came to the opposite conclusion, namely, that it increases the pressor action of adrenaline. The last mentioned authors have also noted that reduction of ventilation brings about diminution of the effect of adrenaline.

The problem is of interest as, besides ordinary changes of respiration which occur throughout life, it is known that in cases of administration of adrenal extracts, or adrenaline, there occur marked reflex changes in respiration which, in turn, may modify the pressor action of these substances.

Consequently, further investigation seemed warranted, and this paper presents the results of a systematic study of the effects of variations in ventilation on the pressor action of adrenaline.

METHODS. Spinal cats under artificial respiration were used. In the majority of the experiments, the Palmer pump was kept at a frequency of 20 to 40 strokes per minute, the variations in ventilation being controlled by the extent of the stroke. Occasionally, in order to produce maximal hyperventilation, both depth and frequency of the strokes were increased.

Adrenaline hydrochloride (Parke, Davis & Co.) in a 1:20,000 solution was introduced into the femoral vein. The doses used were small (0.2 cc. per injection) so as not to cause a maximal rise of blood pressure. The injections were given at regular intervals (Elliott's technique (4) (5)). For reasons mentioned in the text, two successive injections of adrenaline were given with an interval of 1 to $1\frac{1}{2}$ minutes between them; 10 to 15 minutes were allowed to elapse before the next two injections.

Blood pressure records were made from the carotid or femoral artery. In experiments in which the blood gases were analyzed, 3 cc. samples of arterial

blood were withdrawn under paraffin 3 minutes after the second injection of adrenaline. The determination of the blood gases and of the haemoglobin content of the blood was made by the Van Slyke-Neill methods (6).

Various conditions of ventilation are referred to in the text: thus "minimal ventilation" designates the least possible amount necessary to maintain an animal alive over a considerable period of time. Blood gases were: O_2 content from 4.6 to 8.8 vol. per cent; CO_2 from 45.2 to 55.9 vol. per cent in different experiments.

By true "hypoventilation" is meant a state of gradually progressing asphyxiation with a progressive fall of O_2 content of the blood and a rise of CO_2, resulting in convulsions and death of the animals. The term "optimal ventilation" is applied to a state of optimal aeration, the O_2 content of the blood reaching its highest level of from 13.4 vol. per cent to 14.5 vol. per cent, and the CO_2 varying from 35.2 to 42.6 vol. per cent.

A further increase of aeration is referred to as "hyperventilation" of various degrees, characterized by constant values for the O_2 content of the blood (around 14 vol. per cent), but progressive diminution of the CO_2.

RESULTS. The effect of changes in ventilation on the pressor action of adrenaline was observed in more than 30 experiments. Determination of the amounts of gases in the arterial blood, along with records of blood pressure and artificial respiration, was made in 8 experiments. A typical experiment of that kind is presented in table 1.

From this experiment it may be deduced that, in order that adrenaline may exert its maximal effect, both O_2 and CO_2 must be present in the blood in appropriate amounts. Within the range of "optimal ventilation," during which the rises of blood pressure due to adrenaline were greatest, and the augmented effect of the second injection was most pronounced (fig. $A1$), the O_2 of the blood remained at a constant level (around 14 vol. per cent), and the slight variations in the adrenaline effect were apparently caused by changes in the CO_2 content of the blood, which varied from 42.6 vol. per cent to 35.2 vol. per cent.

In the case of "minimal ventilation," two successive injections of adrenaline always evoked small and equal rises of blood pressure (fig. $A2$). The O_2 content of the blood, with this degree of ventilation, was low and ranged between 4.9 vol. per cent and 7.2 vol. per cent; in all the experiments performed similar results were obtained. The CO_2 content of the blood was high and showed considerable variation from animal to animal, ranging from 45.2 vol. per cent to 55.9 vol. per cent.

"Hyperventilation" also caused a decline in the effect of adrenaline, which became obvious when the CO_2 content of the blood fell below 35 vol. per cent, the O_2 values remaining fairly constant—around 14 vol. per cent. As shown in the experiment presented in tabular form, when the CO_2 content of the blood dropped to 27 vol. per cent, convulsions occurred, and the effect of adrenaline applied after cessation of the convulsive seizure was greatly reduced (fig. $A3$). Resumption of optimal ventilation restored the efficacy of adrenaline coincidentally with the increase of CO_2 in the blood.

In many experiments the effects of these variations of artificial respiration could be repeated several times. During the progress of an experiment the haemoglobin content of the blood often fell and the general condition of the animal became poor towards the end.

If hyperventilation was continued over a prolonged period of time irreparable damage occurred after which further changes of ventilation remained ineffective.

In figures B, C and D, a typical result of such a prolonged hyperventilation is shown. Complete cessation of the convulsions and permanent disappearance

TABLE 1

Blood gases in various states of ventilation

NO. OF BLOOD SAMPLE	VENTILATION	BLOOD GASES		HEMO-GLOBIN, G./100 CC. OF BLOOD
		CO₂	O₂	
		vol. p. c.	*vol. p. c.*	
1	"*Minimal ventilation*" (cf. tracing fig. *A*2)	45.2	5.7	14.4
2	Range of "*optimal ventilation*" (cf. tracing fig. *A*1)	42.6	14.2	
3		35.2	14.5	11.7
4	"*Slight hyperventilation:*" effect of adrenaline on blood pressure is less pronounced than under optimal ventilation	32.3	14.2	
5	"*Optimal ventilation*" repeated: full effect of adrenaline restored	37.0	14.3	10.8
6	"*Minimal ventilation*" repeated	48.6	7.2	
	"*Optimal ventilation*" repeated: full effect of adrenaline restored (Blood sample not taken)			
7	*Hyperventilation* to conclusive level (cf. tracing fig. *A*3)	27.0	13.7	10.0
	"*Optimal ventilation*" repeated: full effect of adrenaline restored (Blood sample not taken)			

Experiment. November 17. Two successive injections of 0.01 mgm. adrenaline given every 10 min. and artificial respiration gradually adjusted to the desired levels. Blood samples taken from the carotid artery 3 min. after the 2nd of each 2 injections of adrenaline at the time when the effect of ventilation on the action of adrenaline was typical. (Tracings of the blood pressure changes are not presented for this experiment, as they are similar to the ones shown in fig. *A*.)

of all reflexes occurred after 23 minutes of hyperventilation. The coupled injections of adrenaline administered at that time produced equal, although greatly reduced, rises in blood pressure (fig. 1*B*). After 1½ hours' rest, under "optimal ventilation," the injections of adrenaline were repeated. The rises of blood pressure were still slight, with little difference between the first and second injections, and readjustment of the artificial respiration to the minimal and optimal levels had no effect (fig. 1*C* and *D*).

The loss of reflexes, which was noted after prolonged hyperventilation, suggested the possibility that the varying effects of adrenaline might in some way

be associated with the condition of the spinal centres. Destruction of the spinal cord by pithing, or disconnection of the spinal sympathetic centres from the periphery by means of bilateral removal of the sympathetic chains was
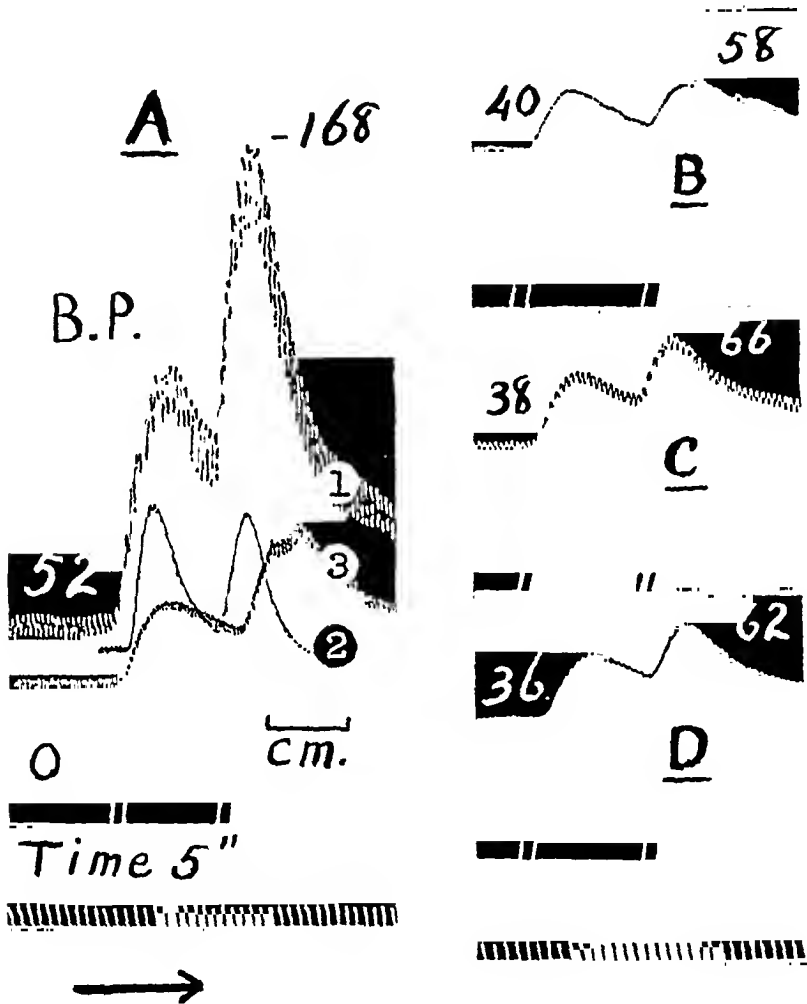
Fig. 1. Expt. Nov. 18. Spinal cat. A—Effects of variations in artificial respiration on the pressor action of 2 successive doses of 0.01 mgm. of adrenaline: 1. Optimal ventilation. 2. Minimal ventilation. 3. Hyperventilation to convulsive level.

B, C and D—Effect of prolonged hyperventilation: B—After 23 minutes of hyperventilation. C and D—Optimal and minimal ventilation repeated after prolonged hyperventilation had caused permanent disappearance of all reflexes. (Effect of pithing of the spinal cord is identical with these tracings obtained after hyperventilation.)

(Abbreviations: B.P.—Blood pressure. (Figures over the blood pressure tracings indicate the height of same in millimeter of mercury.) O—Base line; each two dashes on it show the duration of the intravenous injection of 0.01 mgm. of adrenaline which was timed).

tried. The sensitivity of the preparation to adrenaline decreased markedly after these procedures, and the rises of blood pressure caused by adrenaline remained unaltered by changes in the artificial respiration. (Tracings not pre-

sented, as they closely resemble the effect of prolonged hyperventilation shown in the figure.)   Lowering of the systemic blood pressure to 35–40 mm. Hg by bleeding, adrenalectomy or histamine also decreased the sensitivity of the preparations to adrenaline.   However, changes in ventilation were still effective in these experiments, and only large doses of histamine decreased their effectiveness.   Reintroduction of citrated blood, in the case of bleeding, restored the full effect of adrenaline.

Control experiments were performed in order to exclude the mechanical effects of readjusting the rate and volume of artificial respiration.   The movements of the pump were kept unaltered, the "dead space" being changed according to the technique of McDowell (7).   The results of adrenaline injections in these experiments were similar to those obtained when the respiratory movements were modified.   Controls were also carried out in which anesthetized animals were placed into a decompression chamber and the effectiveness of adrenaline studied under conditions of breathing in rarified atmospheres.   The effect of adrenaline was maximal at ground level and decreased markedly between 10,000 and 20,000 feet above sea level.   Administration of oxygen promptly restored the effectiveness of the adrenaline.

DISCUSSION.   It is evident from the above results that both hypo- and hyperventilation can decrease markedly the pressor action of adrenaline.   As regards hypoventilation, this conclusion is in agreement with the observation of Duzár and Fritz (3).   These investigators, however, were led to believe that hyperventilation increases the effectiveness of adrenaline, whereas the findings presented here are in accord with the results of Balint and Goldschmidt (1) and Brehme and Popovicin (2), who hold that hyperventilation diminishes the action of adrenaline.   It is difficult to account for this discrepancy.   In the present investigation, only 2 out of 18 experiments involving hyperventilation showed that this type of ventilation exerted any beneficial effects on the action of adrenaline.   In both these cases the animals were in poor condition, the circulation was failing and, consequently, the results could not be considered typical.   On the other hand, Duzár and Fritz used doses of adrenaline ten times as large as those employed in the present study: thus the conditions in these two sets of experiments are hardly comparable.   It seems likely that Duzár and Fritz were dealing with maximal rises of blood pressure at the beginning. It is conceivable that, under such conditions, the level to which the blood pressure may rise under the influence of an excessive dose of adrenaline might be affected by hyperventilation in quite a different way.

Balint and Goldschmidt, as well as Duzár and Fritz, contend that the effect produced on the pressor action of adrenaline by changes of ventilation is the result of the direct interaction between the altered *milieu* and that substance.

In the course of this work it was found that loss of blood, histamine, adrenalectomy, pithing and removal of the sympathetic chains caused a marked diminution of the effectiveness of small doses of adrenaline, as well as changes in ventilation.   A common feature in all these experiments is a decline of the arterial blood pressure, and it seems likely that a redistribution of blood in the

circulatory system, which results in a diminished venous return, and consequent reduction of the amount of blood in the arteries, is responsible for the decrease of the effectiveness of adrenaline. The fact that introduction of blood, or Ringer-Locke solution, into the femoral vein, restored the response of the preparation to adrenaline in many of the experiments, favors this supposition.

Henderson (8) and Henderson and Harvey (9) claim, in relation to shock, that changes in the CO_2 content of the blood affect a venopressor mechanism, which controls the flow of venous blood. The effectiveness of adrenaline, in those of our experiments which involved changes in ventilation, was closely associated with the level of the CO_2 and O_2 in the blood, and it is possible that a phenomenon, related to the one suggested by Henderson, is responsible for variations in the action of adrenaline.

However, as the destruction of the spinal cord and other procedures caused a drop in the sensitivity of the preparation to adrenaline, without any changes in the content of gases in the blood, other possibilities, particularly the ones suggested as factors in shock, should be kept in mind. A review of this subject has recently been made by Moon (10). Another important matter, which might have bearing on the problem, is the state of the vasomotor system in anoxia and asphyxia; this was fully discussed in a publication by Gellhorn and Lambert (11).

One more point deserves mention, i.e., the result produced when one injection of adrenaline is followed by another at a short interval. In the case of optimal ventilation, the second injection of that substance always produces a greater rise of blood pressure than the first. That this is a true augmented effect may be judged from several experiments in which the fall of the blood pressure after the initial rise was so rapid that at the beginning of the second injection the blood pressure was at, or below, the resting level. This augmented effect may reasonably be attributed to raised excitability of the neuromuscular apparatus of the circulatory system, caused by the first dose of adrenaline. In conditions of reduced ventilation, this augmented effect might be counterbalanced by delayed recovery of the contractile elements from fatigue due to anoxemia, which would bring about an equalization of the two rises of blood pressure or overswing of the first. However, much more complicated relations may actually exist, and this explanation is merely offered tentatively.

SUMMARY

1. In spinal cats, variation in pulmonary ventilation alters markedly the pressor action of small doses of adrenaline. With both hypo- and hyperventilation, the rises of blood pressure induced by adrenaline are smaller than when ventilation is normal.

2. Bleeding, histamine, adrenalectomy, damage of the spinal cord or bilateral removal of the sympathetic chain result also in reduction of the sensitivity of the preparation to small doses of adrenaline.

3. Prolonged hyperventilation or destruction of the spinal cord abolishes the effects of changes in ventilation on the action of the small doses of adrenaline.

REFERENCES

(1) BALINT, A. AND J. GOLDSCHMIDT.   Jahrb. f. Kinderheilk. **99:** 252, 1922.

(2) BREHME, T. AND G. POPOVICIN.   Ztschr. f. d. ges. exper. Med. **52:** 579, 1926.

(3) DUZÁR, J. AND G. FRITZ.   Klin. Wchnschr. **3:** 2338, 1924.

(4) ELLIOTT, T. R.   J. Physiol. **32:** 401, 1904–05.

(5) ELLIOTT, T. R.   Ibid. **44:** 374, 1912.

(6) PETERS, J. P. AND D. D. VAN SLYKE.   Quantitative clinical chemistry **2:** 283, 321, 337.   Baltimore: Williams and Wilkins Co., 1932.

(7) McDOWELL, R. J. S.   J. Physiol. **71:** 417, 1931.

(8) HENDERSON, Y.   This Journal **27:** 152, 1910–11.

(9) HENDERSON, Y. AND S. C. HARVEY.   This Journal **46:** 533, 1918.

(10) MOON, V. H.   Shock and related capillary phenomena.   Oxford Univ. Press, New York, 1938.

(11) GELLHORN, E. AND E. LAMBERT.   The vasomotor system in anoxia and asphyxia. Univ. of Illinois Press, Urbana, 1939.

EVAPORATION FROM HUMAN SKIN WITH SWEAT GLANDS INACTIVATED

ERNEST A. PINSON[1]

From the Departments of Physiology and Pediatrics, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Total insensible perspiration or insensible loss is the weight lost from the body by evaporation of water from the skin and the respiratory passages and by volatilization of carbon dioxide in excess of oxygen absorbed. Of the components which make up insensible perspiration, that contributed by water loss through the skin is perhaps least understood. Where does cutaneous insensible perspiration arise? Does sweat secretion participate in insensible perspiration; in other words, do the sweat glands secrete constantly, or only in emergency? Aside from the sweat glands, opinions have differed as to the mode of transfer of water through the rest of the skin; transfer might be either secretory or evaporative.

The experiments reported here were designed to determine whether secretion by sweat glands contributes to cutaneous insensible perspiration, and to evaluate physiological and environmental factors that have been supposed to affect cutaneous insensible perspiration. It was conceived that the cutaneous insensible loss might be related to only two variables, namely, cutaneous surface temperature and atmospheric vapor tension.

METHODS. For the collection of moisture emanating from a local skin area, a capsule covering a small area, similar to the one described by Kuno (1927), was employed. Skin temperature beneath the capsule was measured by a thermocouple built into it. Dried and metered air was drawn through the capsule and then through a weighed flask that retained the moisture in concentrated sulfuric acid.

A bellows-type dry meter (fig. 1) indicated the volume flow of air to within 2 per cent. A and A^1 were 500 ml. drying bottles filled with pumice-stone saturated with sulfuric acid. B was a hemispherical glass capsule with a volume of approximately 60 ml. that covered an area of skin, S, of about 20 cm². Perforated glass bulbs, X and X_1, diffused and mixed the air as it flowed through the capsule. C and C^1 were copper and constantan wires passing to two thermojunctions for measuring the skin temperature and the air temperature beneath the capsule. A D'Arsonval galvanometer measured the electromotive force between the thermojunctions and their mates at a known temperature in a thermos bottle. F and F^1 were 60 ml. Erlenmeyer flasks filled with pumice-stone saturated with sulfuric acid for collecting the moisture picked up from the skin beneath the capsule. Many such flasks were placed successively in the circuit for 10-minute intervals, each being weighed before and after. A water manometer, M, recorded the pressure; −20 mm. of water gave a flow of 250 ml. of air per minute. L was a 20-liter carboy serving as an air reservoir to smooth out the rapid, short-period fluctuations in the suction from the negative pressure tap, so that a steady air flow was obtained. The air flow was varied by adjusting the tube E at any

---

desired level below the surface of the oil in bottle H. Steady rates of air flow between 10 and 1000 ml. per minute were thus obtained. For observations of insensible perspiration where the amount of moisture collected did not exceed 0.6 mgm. per minute the rate of air flow was adjusted to 100–150 ml. per minute; the air emerging from the capsule was then less than 20 per cent saturated with water vapor. To dry the rubber tubing a slow stream of dry air was kept flowing through the system over night before each test.

Tests with a second flask for absorbing moisture showed that no detectable amount of vapor (< 3 per cent, at the basal rate) escaped absorption by the first flask. The accuracy obtained by the present method appears to be equal to that reported by Neumann *et al.* (1941). A duplicate of the system shown in figure 1 made possible simultaneous measurements on two areas.

Heavy dark stopcock grease sealed the capsule to the skin without pressing enough to blanch it, and spread outward for approximately 2 cm. The grease prevented evaporation from the small area it covered around the capsule, making possible the visual detection of even very small droplets of sweat at the pores
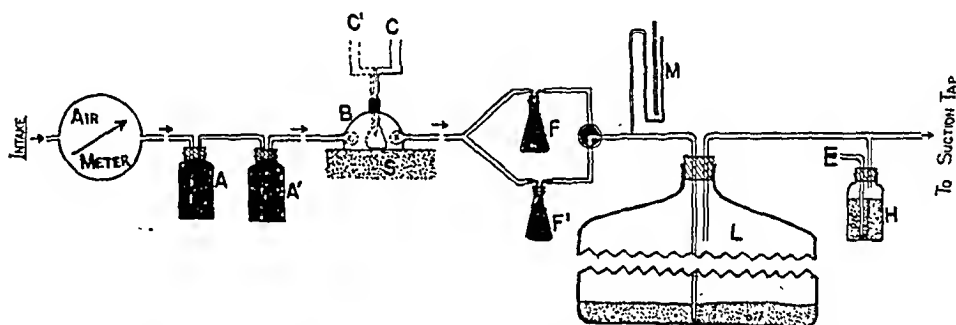
Fig. 1. Diagram of apparatus for measurements of rate of local cutaneous perspiration and of surface temperature. See text for explanation.

under it. The sensitivity of this test of sweat gland activity will be indicated later.

The subjects, clothed only in shorts, reclined on a bed. The room temperature was 28°C. ± 1°C., except where the procedure of the experiment called for alterations in the room temperature. A period of one-half to one hour on the bed was allowed before observations were begun.

Inhibition of the sweat glands and its consequences. Do sweat glands contribute to the evaporation observed on normal body areas under the conditions prescribed above? The plan was to determine simultaneously the rates of evaporation on two symmetrical body areas, then to disable the sweat glands on one of these areas, and to compare the rates of evaporation on the two areas before and after the one was disabled.

To incapacitate the sweat glands, the formaldehyde procedure described by Ichihashi (1936) and Kuno and Ichihashi (1937) was used. A 45 sq. cm. circular piece of blotting paper saturated with 5 per cent formaldehyde was placed on the area selected, and covered with a copper anode of similar shape and size. An indifferent cathode was put elsewhere on the body and a direct current of approximately 0.3 milliampere per cm.[2] of anode flowed for 10 to 15 minutes. This

procedure, when repeated three times at intervals of 2 or 3 days, completely incapacitated the sweat glands on the cataphorized area for a period of 2 to 4 weeks. Ichihashi (1936) and Abramson and Gorin (1940) showed that the sweat glands were probably the chief paths of the cataphoretic current.

Proof of the disability of the sweat glands in treated areas is furnished in figures 2 and 3. The subject was made to sweat profusely on the general body surface by radiations from a 630-watt electric heater mounted in an ordinary copper parabolic reflector suspended 125 cm. above his bed. Previous to the cataphoresis this treatment produced profuse sweating on both legs, whereas after cataphoresis the treated area of the right leg showed no sweat gland activity with the same stimulus, for no sweat droplets could be seen beneath the grease surrounding the capsule and no significant increase in the amount of
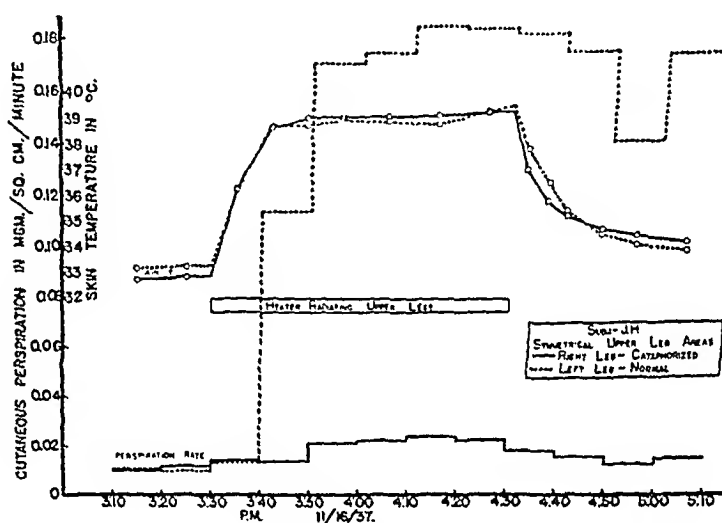
Fig. 2. Responses of skin cataphorized with formaldehyde and of normal skin to local direct radiant heat. The right leg was cataphorized for 10 minutes each day on October 30, November 2, 4, and 6 with 5 per cent formaldehyde beneath the anode at 0.3 milliampere per sq. cm.

evaporation from the cataphorized area occurred (fig. 3). The small increase in moisture collected from the cataphorized area during the heating shown in figure 2 results from the increase in skin temperature. This will be discussed below.

Since the rate of insensible perspiration on the cataphorized area, previous to heating, is no less in general than the rate on the symmetrical non-cataphorized area, it appears that, in the pre-heating stage, activities of sweat glands were contributing nothing to the moisture evaporated from the normal area.

Cataphoresis was carried out in a similar manner on four other subjects and in no instance was insensible perspiration from a cataphorized area significantly decreased thereafter. This was true on arm and chest areas as well as on the legs. While on five of the seven areas cataphorized the rate of insensible perspiration was not significantly altered by the cataphoresis, on two areas where

the cataphoresis was applied more intensely, *i.e.*, by longer applications at shorter intervals, the basal rate of insensible perspiration increased 2 to 3 fold after the treatment although the sweat glands had been definitely disabled. These areas appeared scaly, dry, and irritated, and showed signs of desquamation of the epidermis, in contrast to the less intensely cataphorized areas which appeared like normal skin. The augmented rates of insensible perspiration noted on the scaly areas may possibly be due to the greater ease with which water vapor might escape through the injured skin, or to some increase in activity taking place in replacing the desquamating epidermis. Its temperature did not differ from that of the symmetrical area. Whatever be the cause of this increase in evaporation, the increment of insensible water loss with change of surface temperature on this area is the same as that upon normal areas of skin.
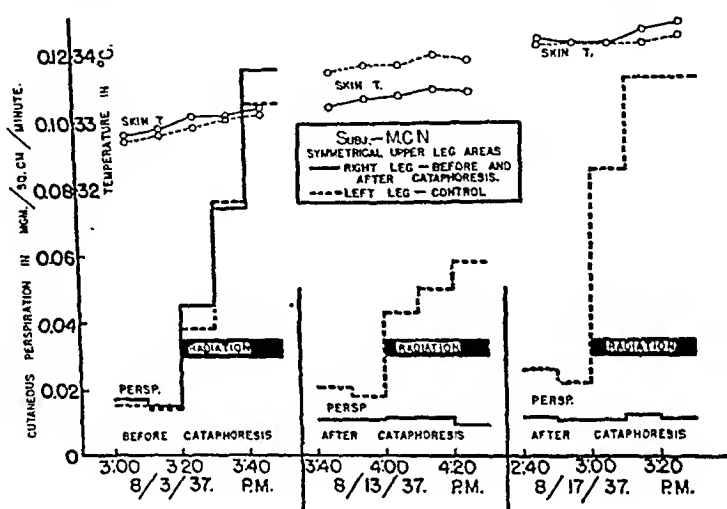
Fig. 3. Response of skin area on leg to radiation on trunk before and after cataphoresis with formaldehyde. The right leg was cataphorized for 8 minutes each day on August 5, 7, and 11, with 5 per cent formaldehyde at 0.2 ma. per. sq. cm.

The relation of skin temperature to cutaneous insensible perspiration. With the sweat glands disabled by cataphoresis as described above, it is possible to study the insensible perspiration over a wide range of skin temperatures without fear of sweat secretion complicating the results obtained. The disability is particularly useful at skin temperatures above 36°C., where sweating almost always interferes with measurement of insensible perspiration on normal skin areas.

Skin temperatures were raised by direct radiation of the areas with the electric heater. The thermocouple which measured temperature beneath the capsule was shielded from direct radiation from the heater by the opaque mountings which held the thermocouple in the capsule. The changes produced by this procedure are exemplified in figure 2. Skin temperatures in other tests were lowered by exposing the subject to a cold room temperature.

Figure 4 shows the results obtained in a series of experiments in each of two subjects, simultaneous measurements being made on the normal and on the
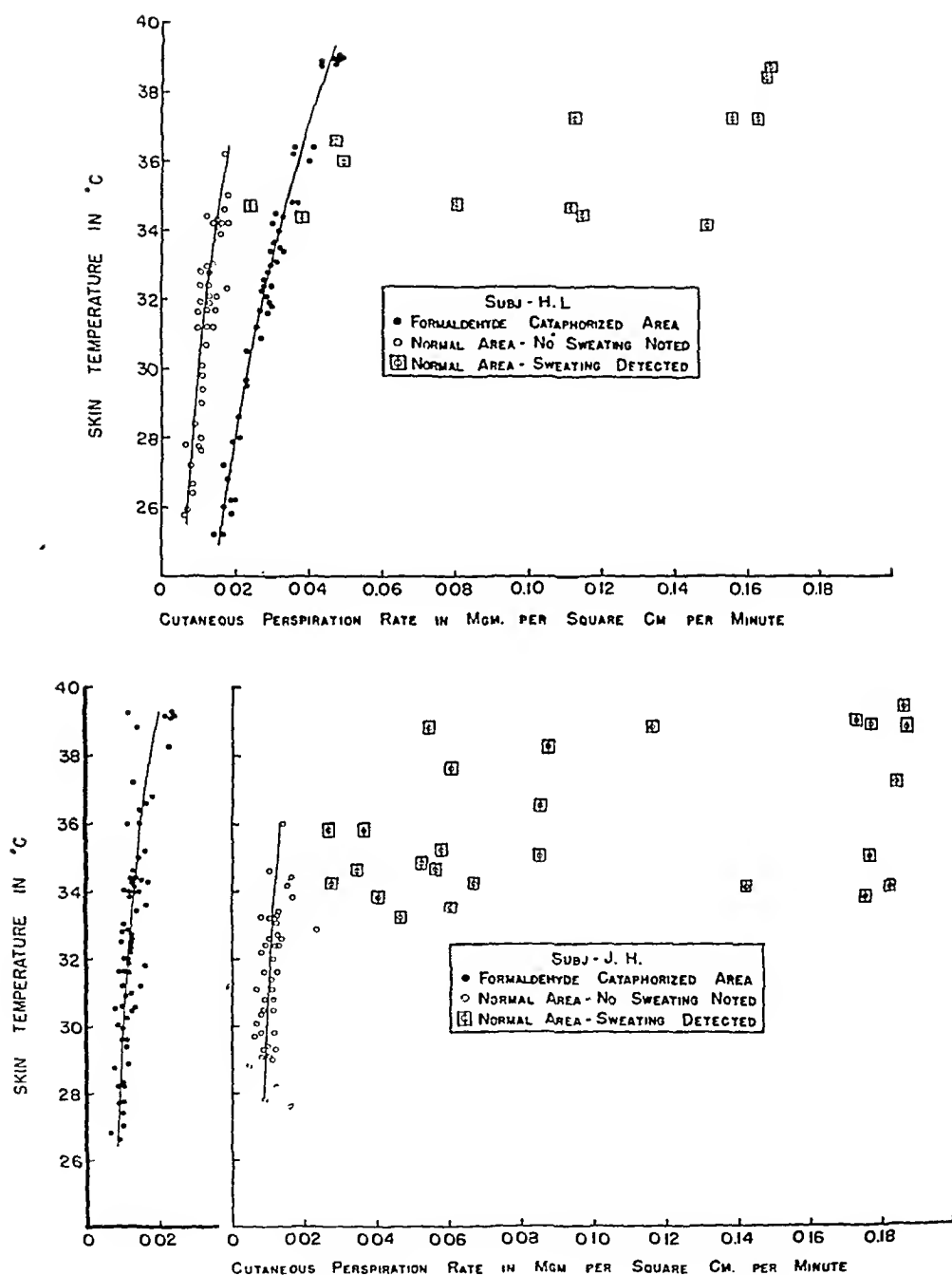
Fig. 4. Relationship between insensible perspiration and surface temperature. Solid dots, cataphorized skin area where sweat glands were disabled. Circles, normal skin area without visible sweating. Squares enclosing circles, normal skin area, but during collection active sweat glands were noted.

cataphorized areas. In the latter the rate of insensible perspiration increased with increase in skin temperature from 25° to 39°C. The increment is such that the insensible perspiration rate is approximately doubled by a 10°C. increase of

skin temperature. The same increment prevailed in normal skin areas, except that when a surface temperature of 34° to 36°C. was reached the sweat glands became active, enormously increasing the rate of evaporation by their secretions.

The sensitivity of the test used (grease over skin) for detection of sweat gland activity is indicated by the plotted data (fig. 4) which show what exceedingly small increments in evaporation rate could be attributed to sweat secretion as judged by the method used for its detection. In general a rate of sweating capable of increasing the evaporation only 0.02 mgm. per cm.² per minute could be detected by the droplets of sweat forming beneath the grease outside the capsule within the 10-minute period of collection.

The fact that the scatter of points (open circles) about the curve for the mean insensible perspiration on the normal area is not much greater than the scatter about the mean on the cataphorized area, and the fact that the two mean curves are quite similar, support the inference that sweat glands on the normal area, as on the cataphorized area, contribute nothing to the evaporation under normal conditions, and wherever the perspiration appears to be insensible as judged by the test used.

It has been noted that in spite of a marked difference in rates of evaporation on cataphorized areas and on normal areas during radiation, there is little difference between the surface temperatures on such areas. This is true whether the areas are being radiated directly (fig. 2) or the radiation is directed at some other body area (fig. 3). It might have been expected that an area on which sweating was inhibited would show a somewhat higher temperature than a similar normal area which was being cooled as a result of evaporation of sweat in more or less profuse quantities. Evidently a differential transport of heat by the blood compensates for the differences of cooling by evaporation.

The relation of peripheral blood flow to cutaneous insensible perspiration. Alterations in peripheral blood flow were induced by subcutaneous injection of drugs, histamine for vasodilatation and pituitrin for vasoconstriction, a few centimeters distal to the capsules from which collections were being made. No significant change in the rate of insensible perspiration followed, although alterations in skin temperatures, sometimes as great as 0.8°C., indicated that the desired variations in peripheral blood flow were being obtained. Figure 5 is typical of the responses to histamine injection. In some cases the pain incident to the injection resulted in an outbreak of sweat secretion on the non-cataphorized area and a consequent transient increase in evaporation from that area.

Blood flow was mechanically obstructed either partially or completely in the arm, by pressure applied in a sphygmomanometer cuff around it (fig. 6). Complete occlusion was produced either by increasing the pressure slowly, in which case the arm was engorged with blood, or by introducing air suddenly into the occluding cuff, in which case the arm contained approximately the usual amount of blood. Partial obstruction resulted when the pressure in the cuff was raised only to a point below systolic arterial pressure so that some blood still flowed into the arm. In eleven experiments on four subjects the rate of insensible perspiration from an arm was not significantly altered either during or after 20

minutes of partial or complete occlusion. In one experiment where slight sweating prevailed previous to the occlusion it was noted that the sweating stopped when the blood flow through the arm was halted. A normal rate of
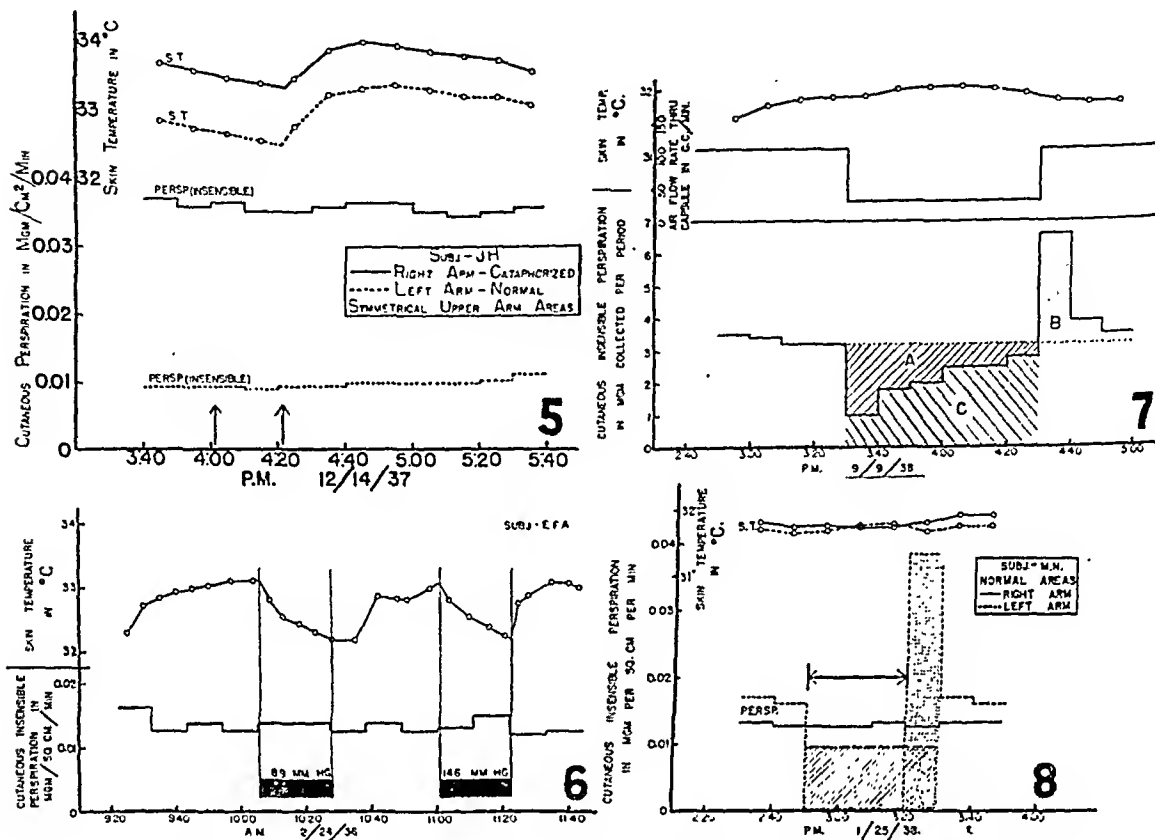
Fig. 5. Responses of surface temperature and of cutaneous insensible perspiration to local injections. One milliliter of 0.8 per cent sodium chloride solution was injected subcutaneously in upper left arm at the first arrow. Four tenths milliliter of 1:1000 histamine chloride solution was injected subcutaneously in each arm approximately 2 cm. distal to each capsule at the second arrow. The flare resulting from the injection spread beneath each capsule.

Fig. 6. Responses of skin temperature and of cutaneous insensible perspiration to partial and complete occlusion. The 89 mm. of mercury pressure did not completely obliterate the pulse at the wrist; the 146 mm. Hg pressure obliterated the pulse.

Fig. 7. Response of cutaneous insensible perspiration to altered rate of air flow through the capsule covering a portion of skin. The geometrical areas designated A, B and C are used in calculations made in table 3.

Fig. 8. Effect of interrupting air flow through the capsule upon cutaneous insensible perspiration. The double arrow designates the 30-minute period during which the air flow was stopped in the capsule on the left arm. The average rate of cutaneous insensible loss for the 40-minute period is also shown (hatched). This area is constructed to equal the stippled area.

insensible perspiration was maintained during and after the occlusion. In two experiments the pain attending readmittance of the blood into the occluded arm was accompanied by the appearance of visible perspiration on the whole

body, with consequent increased evaporation from the area of collection on the arm. When the occluding pressure was released slowly over a period of 1 to 2 minutes, much of the pain encountered during the readmittance of the blood flow was avoided.

TABLE 1

Effect of altered rates of air flow through the capsule upon cutaneous insensible perspiration

1	2	3	4	5	6	7	8	9
							DIMINUTION IN CUTAN. INSENS. PERSP. COLLECTED	
SUBJECT	DATE	ROOM TEMP.	RATE OF AIR FLOW	DURATION OF FLOW	VAPOR PRES. DIFFERENCE	DIMINUTION IN CUTANE-OUS INSENS. PERSP. RATE	Mgm. $(A - B)^*$	Per cent $\dfrac{(A - B)^*}{(A + C)}$
		°C.	ml./min.	min.	mm. Hg			
A. B.	9/7/38	24	110	30	32.2			
			45	60	28.3	11%	1.7	9
			110	30	32.2			
A. B.	9/8/38	24	110	30	33.4			
			38	60	28.6	14%	1.9	11
			110	30	33.4			
A. B.	9/9/38	24	110	40	32.3			
			31	60	26.0	16%	2.2	12
			110	30	32.3			
A. B.	9/3/38	24	110	30	31.6			
			81	30	30.9	9%	} 3.2	14
			35	40	27.3	18%		
			110	30	31.6			

Column 6 shows the vapor pressure difference existing between body fluids at the recorded skin temperature and air emerging from the capsule.

Column 7 shows the per cent diminution in the cutaneous insensible perspiration rate during the last 10-minute period of the diminished air flow, considering the insensible perspiration rate at 110 ml./min. flow to be 100 per cent.

Column 8 shows the diminution in the amount of cutaneous insensible perspiration collected from a 16 cm.² area of the skin as a result of the period of diminished air flow.

* The letters in parenthesis at the head of columns 8 and 9 refer to areas designated in figure 7.

The relation of vapor tension to cutaneous insensible perspiration. What effect has the vapor tension of the air over a local skin area on the insensible perspiration rate from that area? In so far as insensible perspiration may be a physical process of diffusion of water vapor through the skin, an increase in vapor tension in the outside air might be expected to slow the rate of transfer of water through the skin.

In the experiments reported here the rate of flow of dry air through the capsule was varied, so that the vapor tension of the air in the capsule and emerging from

the capsule was altered in accordance with the rate of air flow and the amount of moisture picked up in the capsule. Sometimes the air flow was stopped for a time. Since in these tests the effect of change of vapor tension cannot be divorced from the effect of change in the rate of air flow, both must be considered in any conclusions that are drawn with regard to alterations in insensible perspiration.

Figure 7 exemplifies the results obtained when the rate of dry air flow through the capsule was decreased and later restored to the original rate. A decrease in the rate of air flow reduced the apparent rate of insensible perspiration. This reduction was most marked during the first period of the decreased flow, and although the insensible perspiration increased in subsequent periods of the continuing slow air flow, it never quite reached the level attained with the more rapid flow. When the air flow was restored to its initial rate a washing out of moisture seemed to occur. Calculations reveal that all of the moisture washed out could not have come from stagnant air in the capsule, and hence some emerged from the skin. Possibly the water level in the skin moved nearer to the surface during the periods of reduced air flow and then receded to its initial level upon restoration of the initial air flow. The total excess of moisture removed upon restoration of the initial rate of air flow was not sufficient to account for all the deficit noted during the periods of reduced air flow (table 1). This means that the rate of insensible perspiration was reduced by the slower rate of air flow and the higher vapor tension accompanying it.

The effects obtained by stopping the air flow for a time may be seen in figure 8. The amount of moisture washed out upon resumption of air flow is less than the amount which would have come out during the whole period had the air flow not been changed (3 expts.). While it is clear that insensible perspiration is decreased by a higher exterior vapor tension, the data can hardly be relied on to indicate the quantitative extent of the decrease.

DISCUSSION. Experiments of Kuno and Ichihashi (1937) support the evidence presented here that areas of the skin subjected to repetitive cataphoresis with formaldehyde suffer, for several weeks, complete suppression of sweat secretion in response to heat. That the insensible perspiration is not diminished by this suppression of sweat secretion is clearly indicated by our data; and together the facts seem to justify the inference that no part of the insensible perspiration of normal skin is secreted by sweat glands. Among the modes by which water goes through the skin, fluid might move from the interstitial spaces into the sweat ducts and thus pass through the epidermis by way of the sweat ducts. This possibility seems unlikely in view of our failure to detect any fluid at the sweat pores during insensible perspiration with the method used, although the method was sufficiently delicate to detect droplets at the pores within 10 minutes when sweating was so slight as to increase the evaporation rate only 0.02 mgm. per sq. cm. per minute. Detection of such fluid at the pores seems assured, if it were present, for none evaporates under the grease and consequently it accumulates over a period of 1 to 2 hours. That sweat glands and sweat ducts are not essential to insensible perspiration has also been demonstrated by Loewy and Wechsel-

mann (1911) and Richardson (1926) who found that the normal rate of cutaneous insensible perspiration prevailed in three persons suffering from congenital absence of sweat glands.

That the rate of cutaneous insensible perspiration is conditioned by the temperature of the skin has been inferred by many investigators. Erismann (1875) studied evaporation through dead skin covering water or serum. He found the rate of evaporation to be approximately doubled with a temperature increase of 10°C. This is comparable to the results obtained with normal skin in our experiments. It was Loewy and Wechselmann's opinion that the insensible perspiration was proportional to changes in skin temperature resulting from any cause, and that the reduced evaporation rates which they found on venous obstruction and occlusion could be attributed to lowered skin temperatures. Jores (1930) and others conclude that circulatory variations have no influence on insensible perspiration. Some observers, as Kuno, have noted reductions in rates of evaporation after obstruction of the blood flow which were believed to be too great to be accounted for by the change of skin temperature recorded. No alteration in insensible perspiration occurred in the present tests, during occlusion or other alteration of blood flow, which cannot easily be explained by skin temperature changes. However, it was noted that if slight sweating is prevalent on an arm previous to occlusion, the occlusion will stop the sweating on that arm and the evaporation rate will be reduced considerably thereby. Such a change obviously cannot be considered as a change in *insensible* perspiration, and without a test for sweating, the sweat would not be detected. It is concluded, therefore, that circulatory changes of the sorts observed have no influence on insensible perspiration except in so far as they may alter the skin temperature. The relatively large alterations in cutaneous perspiration noted by some workers during and after occlusion may be attributed to alterations in sensible perspiration.

Two possibilities which would account for the relation noted between skin temperature and cutaneous insensible loss may be postulated. One is that the cutaneous insensible perspiration may be due to some reaction which liberates water near the skin surface, and the other is that the loss of water may result from a process of osmosis or of diffusion through the skin. It is well known that many reactions follow van't Hoff's law over certain temperature ranges, doubling or trebling their rate with each 10°C. temperature rise; such a reaction would explain the relation noted. On the other hand, a process of diffusion dependent on the vapor tension difference between the inside and outside of the skin might equally well fit the relation noted. The vapor tension of the body fluids at 26°C. is approximately 24 mm. Hg, while the vapor tension of the air emerging from the capsule at the rate of flow used in the experiments was approximately 2 mm. Hg. This gives a vapor pressure difference of 22 mm. Hg at a skin temperature of 26°C. At a skin temperature of 36°C. the vapor pressure difference between inside and outside is approximately 41 mm. Hg. Thus, the vapor pressure differences which prevailed over the range studied are sufficient to account for the relation noted between cutaneous insensible perspiration and skin temperature.

If insensible perspiration arises by a process of diffusion of water vapor through the skin, it could be altered by these variations in vapor tension on the exterior of the skin. Presumably a high vapor tension on the exterior would reduce the difference of tension between the inside and the outside of the epidermis, reducing the rate of transfer of water vapor from inside to outside proportionally. If the mechanism is secretory, however, minor alterations in the vapor tension of the outside air might be expected to produce little, if any, effect upon the cutaneous insensible perspiration rate. Actually a decreased ventilation rate through the capsule and the consequent increased vapor tension over the skin resulted in a diminution of the insensible perspiration rate.

Many investigators have studied the relation of total insensible perspiration to environmental humidity, while comparatively few have attempted to relate *cutaneous* insensible perspiration to humidity changes. These latter observers obtained increases in the cutaneous insensible perspiration of man on lowering the humidity (Erismann, Vasti). When loss through the skin compensates for changes of loss through the lungs, it is possible that temperature of the skin also changes (Adachi and Ito).

Whitehouse, Hancock and Haldane (1932) and Trolle (1937) studied the relation of vapor tension difference to exchange of liquid water through the skin. They submerged their subjects, except for the head, in water baths of various salt concentrations. They found that a subject in distilled water at 33° to 34°C. loses no water through the skin or may even gain some. As the salt content of the bath is increased the cutaneous water loss becomes greater until it approaches the rate of insensible perspiration in air. These data indicate that the rate of transfer of liquid water as well as of aqueous vapor through the skin is dependent on a gradient in vapor tension.

The author acknowledges the suggestions of Dr. E. F. Adolph and Dr. S. W. Clausen in this investigation.

SUMMARY

Repeated anodal cataphoresis of formaldehyde into a local area of skin renders the sweat glands in that area non-responsive to heat stimuli for periods of 2 to 4 weeks. Sweating as such can be detected within ten minutes of its initiation, when the sweating is sufficient to increase the evaporation rate only 0.02 mgm. per sq. cm. per minute. When sweat glands are inactivated, liquid water does not come to the skin surface.

Cutaneous insensible perspiration approximately doubles in rate with a skin temperature increase of 10°C.

The rate of blood flow through the skin appears to have no effect on the cutaneous insensible perspiration rate, except as it alters the skin temperature.

An increased vapor tension over the skin, effected by decreasing the rate of air flow over the surface, decreases the rate of cutaneous insensible perspiration.

A process of diffusion of water vapor through cornified layers of epidermis is probably responsible for cutaneous insensible perspiration.

REFERENCES

ABRAMSON, H. A. AND M. H. GORIN. J. Physical Chem. 44: 1094, 1940.

ADACHI, J. AND S. ITO. J. Orient. Med. 21: 881, 1934.

ERISMANN, F. Ztschr. f. Biol. 11: 1, 1875.

ICHIHASHI, T. J. Orient. Med. 25: 101, 103, 105, 107, 1936.

IKEUCHI, K. AND Y. KUNO. J. Orient. Med. 7: 665, 1927.

JORES, A. Ztschr. f. d. gesam. exper. Med. 71: 170, 1930.

KUNO, Y. J. Orient. Med. 7: 497, 1927.

The physiology of human perspiration. Churchill, London, 1934.

KUNO, Y. AND T. ICHIHASHI. Chinese J. Physiol. 2: 41, 1937.

LOEWY, A. UND W. WECHSELMANN. Virchow's Arch. 206: 79, 1911.

NEUMANN, C., A. E. COHN AND G. E. BURCH. This Journal 132: 748, 1941.

RICHARDSON, H. B. J. Biol. Chem. 67: 397, 1926.

TROLLE, C. Skand. Arch. Physiol. 76: 225, 1937.

VASTI, A. This Journal 102: 60, 1932.

WHITEHOUSE, A. G. R., W. HANCOCK AND J. S. HALDANE. Proc. Roy. Soc. of London, Ser. B, 111: 412, 1932.

MOLYBDENUM IN THE NUTRITION OF THE RAT[1]

J. D. TERESI, C. A. ELVEHJEM AND E. B. HART

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

In recent years the biological importance of molybdenum, particularly with reference to the nutrition of lower plant forms, has been recognized. Steinberg (1) found molybdenum essential for the growth and sporulation of *Aspergillus niger*. Bortels (2) found that sand cultures of peas, soy beans and red clover showed an increase in nitrogen fixation and growth with carefully regulated additions of molybdenum and vanadium compounds.

Further advance in the study of the importance of molybdenum in higher plants has been the result of the work of Arnon and Stout (3), who have found that molybdenum, in minute amounts, improved the growth of barley plants in a culture solution supplied with ammonium salts as the sole source of nitrogen. They also found that a group of heavy metals including molybdenum increased the growth of lettuce and asparagus. The work of these authors showed the essential character of molybdenum in the nutrition of tomato plants and the ability to prevent the deficiency symptoms described in their paper.

Of interest also is the wide distribution of molybdenum in biological materials. Ter Meulen (4) by means of a colorimetric method found large quantities of molybdenum in the liver of the ox and pig (1.5 mgm. per kgm. of liver), but found smaller amounts in such materials as blood, bile, milk, eggs and certain other tissues. These contained 0.03 to 0.14 mgm. per kilo. The paper of Ter Meulen does not state whether the determinations are on a dry or wet basis. Drea (5) by the use of the spectrographic method detected molybdenum in cow's milk. Later Drea (6) demonstrated the presence of molybdenum in eggs, brain, eyes, gizzard, kidney and the liver of hens.

As far as we are aware there is no record in the literature of an attempt to determine whether or not molybdenum is an essential nutrient for animals. Since, as described in the above paragraphs, this element is required by some lower forms and also by some higher forms of plants for normal growth and development, we thought it worth while to investigate its bearing on the nutrition of the rat. Our results on this problem are recorded in this paper.

EXPERIMENTAL. Marmoy (7) has described a method for molybdenum determination involving the extraction of the orange thiocyanate complex with ether, and using the optimum conditions for color development as suggested by Hurd and Allen (8). We have applied this procedure to the determination of molybdenum in biological materials. The ashing conditions and the color com-

parisons which were used were essentially the same as those described by Marmoy with the exception that the color was measured in an Evelyn colorimeter making use of a special 470 mμ filter constructed by the Rubicon company.

TABLE 1

Molybdenum content of whole rats, cow's milk and goat's milk

	WEIGHT (FUR AND SKIN REMOVED)	AGE	GAMMA MO PER KILO (WET BASIS)
Whole rats on goat's milk			
	gms.	*weeks*	
4 weeks on goat's milk—previously on stock diet...........	148	12	81
7 weeks on goat's milk—started on milk at age of 21 days..	125	10	52
5 weeks on goat's milk—started on milk at age of 14 days.....	44	7	56
Whole rats on goat's milk plus 30 gamma of Mo. per day			
Fed Mo since 21 days old	80	7	125
Fed Mo since 21 days old	70	7	107
Fed Mo since 21 days old	70	7	106
Fed Mo for 3 days previous to analysis	36	5	117
Average...			114

	SAMPLES (100 CC.)	MICROGRAMS MO PER LITER OF MILK
Cow's milk	1	41
	2	40
	3	58
	4	42.7
	5	56
Average.........................		47.5
Goat's milk	1	15
	2	16
	3	16
	4	12
	5	12
	6	12.5
	7	11
Average.........................		13.5

The ether extraction of the colored complex was carried out 5 minutes after the development of the color.

The standard curve obtained by plotting the log values (densities) of the colorimeter readings against the corresponding known quantities of molybdenum approximated a straight line.

Molybdenum analysis. Drea (9) failed to detect molybdenum in goat's milk

by using a spectrographic procedure. However, he was able to demonstrate the presence of this element in cow's milk. The above fact led us to assume

TABLE 2

Growth of rats on a basal diet of mineralized goat's milk with and without added molybdenum as Sodium Molybdate

	MALES (GRAMS PER WEEK, 6-WEEK PERIOD)	FEMALES (GRAMS PER WEEK, 6-WEEK PERIOD)
Basal*	25	23
	26.6	19
	25.3	19.6
Average..............................	26	21
Plus 30 gamma of molybdenum per 75 cc. of drinking water*	24	22
	30	17
	21	20
	27	23
	26.5	18
	26	
Average..............................	26	20.5
Basal	21	
	20	
	20	
	25	
	21	
	22	
	26.5	
	19	
Average..............................	21.7	
Plus 30 gamma of molybdenum per 75 cc. of drinking water	21.5	
	26.5	
	24	
	21	
	22	
	29	
	22	
Average..............................	23	

* Rats in these groups were obtained from Sprague-Dawley. Other animals were from the stock colony of this laboratory.

that goat's milk might be low enough in molybdenum to warrant its use in the study of this problem.

A series of molybdenum determinations on cow's milk as well as goat's milk

was made. The results of these analyses are given in table 1. The analysis of the two types of milk showed that, although molybdenum was contained in each, the goat's milk contained about one-third as much of this element as did cow's milk. The average value obtained for goat's milk was 13.5 micrograms per liter. The value for cow's milk was 48 micrograms per liter.

Results of the molybdenum content of whole rats (wet basis) are recorded in table 1. Three of the rats analyzed weighed 150 grams and had been on a diet of goat's milk plus 30 micrograms of molybdenum per day since weaning (a period of six weeks). Another rat of the same weight had received the same diet, but the molybdenum was fed for only 3 days (just before analysis). Another group of rats had been on a diet of goat's milk only. Two of these were taken at the age of 21 days from mothers that had been on goat's milk since the young were 14 days old. These rats were kept on the milk diet for six weeks after weaning before they were analyzed for molybdenum. Another rat which had been on the stock ration for 5 or 6 weeks was placed on goat's milk alone for 4 weeks before analysis. In the analysis of the whole rat the G. I. tract and the skin of each animal had been removed.

Molybdenum in the nutrition of the rat. Six litters of rats at the age of 2 weeks were placed with their mothers in zinc cages. The mother rats were kept on goat's milk to reduce the store of molybdenum in the young. The milk was mineralized with iron, copper and manganese. Each animal was given daily 500 γ of Fe, 50 γ of Cu and 50 γ of Mn. After weaning, the young were started on a basal ration of mineralized whole goat's milk with and without the addition of molybdenum. All animals received 2 drops of haliver oil each week. The milk used in this experiment was received in porcelain pails. The growth rate of these rats is shown in table 2.

Assuming a daily intake for a growing rat[2] to be 40 cc. of milk, the animals received 0.5 to 0.6 microgram of molybdenum per day through their ration of milk. The rats, which were receiving molybdenum as sodium molybdate in their drinking water, showed no better growth rate than the rats on the low molybdenum ration.

DISCUSSION. The molybdenum content of whole rats was found to be low when fed goat's milk alone, while it increased somewhat when the milk was supplemented with molybdenum. The body increase, however, was relatively low indicating either a very rapid excretion or low absorption of this element. Since rats, which had been on a dry stock ration, still contained 81 micrograms of molybdenum per kilo of fresh weight after a period of 4 weeks on goat's milk, it may be concluded that the small increase in molybdenum content when this element was added to the milk diet was due to poor absorption of molybdenum.

The values which we obtained for the molybdenum content of goat's milk were not in quantitative agreement with Drea's negative values. The fact that the molybdenum content of this product is low might explain the negative

---

[2] Growth records were taken for a period of 6 weeks after weaning. The weight of each rat at weaning was about 30 grams.

results obtained by the above author, who used a spectrographic method of analysis.

Weanling rats placed on a mineralized goat's milk ration showed comparable growth to litter mates receiving added molybdenum as sodium molybdate.

About 0.5 microgram of molybdenum per day was taken in by a growing rat on a basal ration of goat's milk mineralized with copper, iron, and manganese. Since supplementing this ration with molybdenum did not accelerate the growth rate, it can be concluded that if molybdenum is needed by the growing rat, then 0.5 microgram per day satisfies this requirement.

SUMMARY

1. A colorimetric method for the determination of molybdenum in biological materials has been described. Use of the Evelyn colorimeter was made with the aid of a special filter.

2. Cow's milk contained about 3 times as much molybdenum as goat's milk.

3. Molybdenum fed as sodium molybdate was very poorly absorbed by the rat.

4. The addition of molybdenum to goat's milk did not produce increased growth. A daily intake of 40 cc. of this milk (the usual amount required) contained approximately 0.5 microgram of molybdenum. It can be concluded that if molybdenum is needed by the growing rat then this amount of molybdenum per day satisfies its requirement.

REFERENCES

(1) STEINBERG, R. A. J. Agric. Research 59: 731, 1939.
(2) BORTELS, H. Arch. Mikrobiol. 8: 13, 1937.
(3) ARNON, D. I. AND P. R. STOUT. Plant Physiol. 14: 599, 1939.
(4) TER MEULEN, H. Nature 130: 966, 1932.
(5) DREA, W. F. J. Nutrition 8: 229, 1934.
(6) DREA, W. F. J. Nutrition 10: 351, 1935.
(7) MARMOY, F. B. J. Soc. Chem. Ind. 58: 275, 1939.
(8) HURD, L. C. AND H. O. ALLEN. Ind. Eng. Chem. 7: 396, 1935.
(9) DREA, W. F. J. Nutrition 16: 325, 1938.

PROTHROMBIN ACTIVITY DURING PREGNANCY AND LACTATION[1]

J. B. FIELD, R. S. OVERMAN AND C. A. BAUMANN

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

The present study deals with the effect of the anticoagulant[2] 3,3'-methylenebis(4-hydroxycoumarin) (1) on the prothrombin activity of rat plasma during pregnancy and lactation. It has been reported that the prothrombin activity of plasma is usually increased during the later stages of pregnancy (2–5) but similar increases have not been recorded for the period of lactation. However, it now appears that one of the differences between the lactating and the non-lactating rat is that the former possesses a particularly efficient mechanism for counteracting influences which tend to lower prothrombin activity.

METHODS. The basic procedure was essentially that employed previously (6). Adult rats 150 grams in weight or over were fed an artificial diet of the following composition: casein 18, yeast 8, salts (Wesson) 4, cod liver oil 2, and dextrin 68. A hypoprothrombinemia was induced as follows: the rats were starved for 12 hours and then fed 2.5 mgm. of the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) incorporated into 2 grams of the ration which was consumed within 30 minutes. Four hours later the ration itself was fed ad libitum, and 24 hours after the ingestion of the anticoagulant, blood samples were taken by heart puncture. The prothrombin time of 12.5 per cent plasma was determined by a standard procedure (7).

The extent of the hypoprothrombinemia induced was determined in 60 rats in various stages of pregnancy and lactation and in the same animals after lactation had ceased. Included were 14 rats whose period of lactation was extended artificially for periods up to 180 days. Numerous non-lactating rats of both sexes served as controls. Since an interval of one week was allowed between heart punctures in any one animal, the number of determinations made during a normal period of pregnancy and lactation seldom exceeded five. The procedure was sufficiently mild for the young to be born and raised without visible abnormality. Pregnancy was determined by routine examination of the vaginal smears.

EXPERIMENTAL. *Prothrombin time in pregnancy and lactation.* The prothrombin time of 12.5 per cent plasma from non-pregnant rats on the artificial ration ranges from 36 to 45 seconds, average 40 (6) and this range was also

---

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] The term anticoagulant is used in the general sense that 3,3'-methylenebis(4-hydroxycoumarin) is an agent, which after action *in vivo*, impairs or prevents the coagulation of blood. It does not affect clotting power when added to blood or plasma *in vitro* (7) p. 12. We are indebted to Prof. K. P. Link for the 3,3'-methylenebis(4-hydroxycoumarin) used in these investigations.

observed during lactation and during the first two weeks of pregnancy. However, during the last week of pregnancy the prothrombin time ranged from 27 to 39 seconds, average 32. While the difference in averages appears small, it probably represents a very considerable increase in prothrombin activity, since the relation between the prothrombin clotting time of plasma and prothrombin concentration is not linear, and over this particular range relatively small variations in clotting time are indicative of large changes in the concentration of prothrombin (7). In humans an increase in prothrombin activity at the end of pregnancy appears to be well established (2–5).

Induced hypoprothrombinemia. The ingestion of 3,3'-methylenebis(4-hydroxycoumarin) by the non-pregnant non-lactating rat results in a marked hypoprothrombinemia: 2.5 mgm. of the anticoagulant increased the prothrombin time of 12.5 per cent plasma from the normal value of 40 seconds to an average

TABLE 1

The effect of pregnancy and lactation on the hypoprothrombinemia induced in rats fed 2.5 mgm.
of 3,3'-methylenebis(4-hydroxycoumarin)

(Prothrombin clotting time of 12.5 per cent plasma)

	NO. OF DETERMINATIONS	PROTHROMBIN TIME, AVERAGE + S.D.
Non-pregnant	130	112 ± 18
Pregnant first 2 weeks	14	90 ± 23
Pregnant third week	13	81 ± 18
Lactating first week	13	66 ± 18
Lactating second week	13	59 ± 10
Lactating third week	15	53 ± 8
Lactating 4th–8th week	35	58 ± 10
Lactating 14th–25th week	8	61 ± 10
Post-lactating	26	111 ± 25

S.D. = standard deviation.

of 112 (table 1), an increase of 72 seconds. When this amount of anticoagulant was fed to rats during the last week of pregnancy, the prothrombin time ranged from 56 to 107, average 81 seconds (table 1), an increase of 49 seconds. This relative mildness of the induced hypoprothrombinemia might have been an expression of the high prothrombin activity of the plasma before the anticoagulant was administered.

The lactating rat, however, exhibited a resistance to the anticoagulant which could not be attributed to a high initial prothrombin activity. Thus, when 2.5 mgm. of anticoagulant were fed during the third week of lactation, the prothrombin time of 12.5 per cent plasma increased to only 53 seconds in lactating rats as compared to an average of 112 seconds in those not lactating. Since, in the absence of anticoagulant, the prothrombin times of both groups averaged 40 seconds, 36–45, this indicated an increase in the lactating animals of only 13/72, or 18 per cent of the normal increase in seconds. A marked resistance

of lactating rats was observed at all levels of anticoagulant fed, although variations within groups were fairly wide (table 2).

The lactating rats appeared to have a high capacity to recover from incipient hypoprothrombinemia. During the first 12 hours after the ingestion of 2.5 mgm. of anticoagulant there was a definite increase in the prothrombin times, which averaged 61 seconds in 8 lactating rats as compared to 75 seconds in 22 non-lactating controls (6). During the second 12-hour period, however, the difference between lactating and non-lactating animals became very evident. In the lactating rats the average prothrombin time decreased from 61 seconds to 58 seconds; in the non-lactating rats it increased from 75 seconds to an average of 112 seconds. In fact, the response of the lactating rat to the anticoagulant was very similar to that of ordinary rats fed large amounts of vitamin K (6).

The greatest resistance to the anticoagulant, lowest prothrombin time, was observed during the third week of lactation (table 1) i.e., when the secretion of

TABLE 2

The degree of hypoprothrombinemia induced in lactating and non-lactating rats by 3,3'-methylenebis(4-hydroxycoumarin)

(Prothrombin clotting time of 12.5 per cent plasma)

	NO. OF DETERMINATIONS	MGM. OF ANTICOAGULANT	PROTHROMBIN TIME, AVERAGE ± S.D.
Lactating and non-lactating...............	115		40.0 ± 3.2
Lactating................................	83	2.5	59 ± 11
Non-lactating............................	130	2.5	112 ± 18
Lactating................................	6	5.0	62 ± 7
Non-lactating............................	9	5.0	171 ± 35
Lactating................................	4	7.5	97 ± 19
Non-lactating............................	4	7.5	191 ± 42

milk was probably at its greatest volume (8). The connection between lactation and resistance to hypoprothrombinemia was further emphasized by the performance of eight females which either failed to lactate, or stopped lactating shortly after parturition, so that the young died. The administration of anticoagulant to these animals resulted in prothrombin times of 97 to 167 seconds, essentially the range for other non-lactating animals in the post-lactation period. The response to the anticoagulant immediately after withdrawing the young was often temporarily irregular, but ultimately the degree of hypoprothrombinemia induced in all animals was that characteristic of non-lactating rats.

Prolonged lactation. While young rats are usually weaned about the twenty-first day of life, Long and Evans state that the mother may be kept lactating for periods up to 40 days (9). In the present experiment 7 rats were kept in lactation for more than 60 days, while 3 others lactated for more than 110 days. This was accomplished by foster nursing as follows: after the natural litter had opened its eyes, the mother and young were kept in a cage without access to food, the mother being fed the artificial ration in a separate cage during two

30-minute intervals daily. When the young were about 15 days old, all but one or two were removed and replaced with young[3] approximately 5 days of age. As these grew up, the older pups were removed periodically, and replaced with younger ones. The new foster young were seldom neglected if the female was allowed to keep one or two of the older ones to which she had become accustomed. The young either grew or maintained their weights, and their stomachs were visibly distended with milk, thus indicating substantial lactation. A typical protocol is indicated in table 3.

The treatment of the females during the prolonged lactation period was by no means optimal for general well-being: they were fed an artificial ration; they

TABLE 3

Prolonged lactation: protocol of rat 240 for period 100—130 days post-partum

	YOUNG ADDED	YOUNG REMOVED	WEIGHTS OF LITTER*	
			grams	
10-13-41	5 born			
1-21-42	2 11-day old			53
1-22-42	2 13-day old	1 12-day old†	57,	78
1-25-42	2 6-day old	1 28-day old	80,	70
1-27-42	2 7-day old	1 20-day old	76,	79
1-29-42		1 9-day old†	84,	78
1-30-42	2 9-day old	2 21-day old	78,	60
1-31-42		1 21-day old		
		1 12-day old†	64,	44
2- 1-42	2 1-day old		50,	60
2- 3-42	2 12-day old		70,	95
2- 7-42		2 7-day old†	114,	100
2-12-42	1 10-day old	1 23-day old		
	1 16-day old	1 24-day old	117,	98
2-14-42		2 24-day old	105,	60
2-20-42			88,	

* The first figure indicates litter weight before the recorded change in animals; the second figure, litter weight after the change. Growth is indicated by comparing the second figure of any line with the first figure of the next line.

† Died.

were starved periodically for periods of 12 hours; they were given the anticoagulant periodically; and finally numerous blood samples were taken by heart puncture. Nevertheless, the females remained resistant to the anticoagulant throughout the period of prolonged lactation. Thus the ingestion of 2.5 mgm. of anticoagulant by lactating females 100 days after parturition resulted in prothrombin times of only 56 seconds (45–67) as compared to 112 seconds average in non-lactating controls. When the young were permanently withdrawn, however, this amount of anticoagulant induced a degree of hypoprothrombinemia characteristic of non-lactating rats.

[3] We are indebted to Prof. H. Steenbock and to Messrs. H. Gottlieb and F. A. Kummerow for many of these animals.

Liver size during pregnancy and lactation. No satisfactory explanation is as yet at hand for the resistance of the lactating rat to the anticoagulant 3,3′-methylenebis(4-hydroxycoumarin). Prothrombin is believed to be synthesized in the liver (10) and it has been suggested that the anticoagulant interferes with this synthesis. Accordingly, the observed resistance to the anticoagulant might have been due to a greater efficiency of hepatic function during pregnancy and lactation. Poo et al. (11) have reported an increase in liver size during pregnancy, with the liver still somewhat larger than normal after 31 days of lactation. A survey of 17 pregnant rats, and 35 of our lactating ones, revealed an increase in liver size (fresh weight) of approximately 30 per cent during the last week of pregnancy (table 4) with a further increase during lactation. Two animals which died at parturition from uncontrollable hemorrhage had relatively

TABLE 4

Liver size during pregnancy and lactation*

	NO. OF RATS	FRESH WEIGHT OF LIVER
		grams
Normal females		
150–200 grams	11	6.9 (6.2– 7.2)
200–250	9	7.1 (5.9– 8.5)
250+	16	8.4 (6.8– 9.4)
Pregnant		
0–13 days	6	7.8 (6.0– 9.4)
14–21 days	11	10.0 (7.0–13.2)
Lactating		
0–10	15	10.1 (7.4–12.5)
11–20	6	11.6 (9.3–13.7)
21–30	10	11.3 (8.0–13.5)
30+	4	11.6 (8.7–15.2)
Post lactating		
7–14 days	7	8.9 (5.4–11.8)

* In the non-pregnant non-lactating rats the liver constituted 3.4 per cent of the body weight; in the lactating rats, the liver weights averaged 4.5 per cent of the body weight.

small livers, as did 3 sacrificed from a group of 8 post-partum females which failed to suckle their young. Thus the rapid recovery from incipient hypoprothrombinemia during lactation seems to have been associated with an increased liver size during this period. The variations in liver size, however, were hardly of sufficient magnitude to account for more than a fraction of the resistance to induced hypoprothrombinemia exhibited by lactating rats.

DISCUSSION. It is still an open question whether resistance to the anticoagulant 3,3′-methylenebis(4-hydroxycoumarin) is enhanced during lactation in species other than the rat. Extensive reports on the hemorrhagic sweet clover disease in cattle seldom contain references to the sex of the affected animals, although both Schofield (12) and Roderick (13) have observed cows which consumed anticoagulant (spoiled hay) and remained well after parturition, while

the calves died of hemorrhages.   This might suggest not only a resistance on the part of the mother, but also the transmission of anticoagulant through the placenta and mammary gland.   The resistance to the anticoagulant might also be connected with the relatively large turnover of water and other substances during lactation, with the lactogenic hormone a governing factor.

SUMMARY

1. The prothrombin activity of rat plasma was relatively high during the last week of pregnancy, but after parturition it decreased to its former level.

2. The hypoprothrombinemia induced by the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) was somewhat milder in pregnant rats than in those not pregnant.   In lactating rats the anticoagulant was only 18 per cent as effective as in non-lactating controls.   The resistance of the lactating rat appeared to be due to an enhanced capacity to recover from incipient hypoprothrombinemia.

3. By means of foster young, female rats were kept lactating for periods up to 180 days.   Resistance to the anticoagulant persisted throughout lactation.   In the post lactation period, however, a marked hypoprothrombinemia could be readily induced by the anticoagulant.

4. Recovery from induced hypoprothrombinemia appeared to be associated with increases in liver size.   Some increase in liver weight was noted during pregnancy with further increases during lactation.

REFERENCES

(1) STAHMANN, M. A., C. F. HUEBNER AND K. P. LINK.   J. Biol. Chem. **138**: 513, 1941.
(2) THORDARSON, O.   Nature **145**: 305, 1940.
(3) DAM, H., H. LARSEN AND P. PLUM.   Ugeskift f. Laeg. **103**: 257, 1941.
(4) NORRIS, R. F. AND M. C. BENNETT.   Surg., Gynecol. and Obstet. **72**: 758, 1941.
(5) JAVERT, C. T. AND C. MACRI.   Am. J. Obstet. and Gynecol. **42**: 415, 1942.
(6) OVERMAN, R. S., J. B. FIELD, C. A. BAUMANN AND K. P. LINK.   J. Nutrition **23**: 589, 1942.
(7) CAMPBELL, H. A., W. K. SMITH, W. L. ROBERTS AND K. P. LINK.   J. Biol. Chem. **138**: 1, 1941.
(8) COX, W. M., JR. AND A. J. MUELLER.   J. Nutrition **13**: 249, 1937.
(9) LONG, J. A. AND H. M. EVANS.   The oestrus cycle in the rat.   Memoirs of the Univ. of California 6, 1922.
(10) SMITH, H. P., E. D. WARNER, AND K. M. BRINKHOUS.   J. Exper. Med. **66**: 801, 1937.
(11) POO, L. J., W. LEW AND T. ADDIS.   J. Biol. Chem. **128**: 69, 1937.
(12) SCHOFIELD, F. W.   J. Am. Vet. M. A. **64**: 553, 1924.
(13) RODERICK, L. M. AND A. F. SCHALK.   North Dakota Agric. Expt. Sta. Bull. 250, Tech. 1931.

PROPHYLACTIC TREATMENT OF EXPERIMENTAL RENAL HYPERTENSION WITH RENIN[1, 2]

G. E. WAKERLIN, C. A. JOHNSON, E. L. SMITH, W. G. MOSS AND J. R. WEIR

From the Departments of Physiology and Physiological Chemistry, University of Illinois College of Medicine

Two of us (1) have previously reported that daily intramuscular injections of partially purified hog renin for four months produced striking reductions in the blood pressure of dogs with renal hypertension, whereas heat-inactivated hog renin and active dog renin were without antipressor effect. The serums of the dogs treated with hog renin, but not the serums of the dogs given injections of inactivated hog renin or dog renin, neutralized the acute pressor effect of renin (antirenin). The mechanism of these therapeutic effects of hog renin in experimental renal hypertension in the dog, we stated, was not clear, although an antihormone or immune response to heterologous hog renin might be involved.

In view of these significant therapeutic effects of partially purified hog renin which are being substantiated by work now in progress, we have studied the prophylactic effects of hog renin, inactivated hog renin, dog renin, rabbit renin, inactive human renin, and an extract of liver in experimental renal hypertension in the dog. Thus far attempts by other workers to prevent the development of experimental renal hypertension have been unsuccessful.

METHODS. Normotensive dogs were observed during a control period of two to four months. Mean blood pressure readings were obtained by puncture of a femoral artery (method of Dameshek and Loman (2)) two or three times a week. Studies on the blood urea nitrogen, urinalyses, and determinations of the body weight were made at monthly or bimonthly intervals and more frequently when indicated. The dogs were then treated with daily intramuscular injections of renin for approximately six months. In the middle of this period the right and left renal arteries were constricted three weeks apart by the Goldblatt technique (3). The renin solutions used were equivalent to 1 gram of fresh kidney cortex per cubic centimeter of solution and were administered in a dose of 1 cc. per kilogram of body weight. The method used for the preparation of the renin solutions was essentially that described by Grossman (4), except that acetone was employed as a dehydrating agent and much of the associated protein was removed by isoelectric precipitation. The renin solutions were *only partially purified and obviously contained substances other than renin.* Four dogs were treated with hog renin, four with hog renin inactivated by heating at 70°C. for one-half hour, four with dog renin, two with rabbit renin, two with inactive

human renin (renal extract prepared like renin but pressor inactive), and three with liver extract prepared after the manner of renin.

Blood serums were examined for antirenin before treatment and subsequently during treatment at semimonthly and after treatment at monthly and bimonthly intervals. The technic previously described (5) consisted essentially in mixing 2 volumes of serum with 1 volume of renin solution, allowing the mixture to stand at least overnight at 4°C. and assaying the acute pressor effect of the mixture intravenously on the etherized, nephrectomized dog. The dose of renin solution employed was 0.25 cc. per kilogram of assay animal. In all instances the serum tested for antirenin was suitably controlled with serum from untreated normotensive dogs and frequently with serum from untreated hypertensive dogs. Antirenin titers were regularly determined for dog renin, less frequently for hog renin, and exceptionally (two dogs receiving rabbit renin) for rabbit renin.

Determinations of blood pressure, studies on the blood urea nitrogen, urinalyses, and determinations of body weight were continued throughout the treatment period and subsequently during observation periods which varied from one to eleven months.

RESULTS. *Hog renin.* During three months of hog renin injections prior to constriction of the renal arteries, the blood pressures of the four dogs showed no significant change from the normotensive levels observed during the preceding control period of two to three months. Following constriction of the first renal artery there was no significant change in the blood pressures of any of the four dogs. Subsequent to constriction of the second renal artery two of the dogs showed no important change in blood pressure during the remaining three months of treatment with hog renin or during observation periods of nine and eleven months respectively following renin therapy. The results for one of these two dogs are illustrated by figure 1. The third dog showed a significant rise in blood pressure following constriction of the second renal artery. This mild hypertension remained unchanged during the subsequent three months of continued hog renin injections. There was a gradual increase in the hypertension during the five months of observation subsequent to treatment. Following constriction of the second renal artery the fourth dog developed hypertension which persisted essentially unaltered for the five months of observation subsequent to the injections of hog renin (fig. 2).

Antirenin, tested against dog and hog renins, became demonstrable in the serums of the four dogs during the second month of treatment and disappeared during the second month after treatment was discontinued. At no time during treatment or subsequently was there any clinical evidence of untoward effects. The appetites of the four dogs remained excellent, their weights constant, and their blood urea nitrogens and urines normal throughout.

Inactivated hog renin. During the three months of inactivated hog renin injections prior to constriction of the renal arteries, the blood pressures of the four dogs remained unchanged from the normotensive levels noted during the initial control period of two and one-half months. One dog continued to show normo-

tension despite bilateral renal artery constriction. Normotension persisted in this animal during three months of continued inactivated hog renin injections and five months of subsequent observation. Following constriction of the first renal artery, hypertension developed in the three remaining dogs and was intensified somewhat by subsequent constriction of the second renal artery. No significant changes occurred in the hypertensions of these three dogs during the ensuing three months of continued inactivated hog renin injections or during the five months of observation following the completion of the injections.
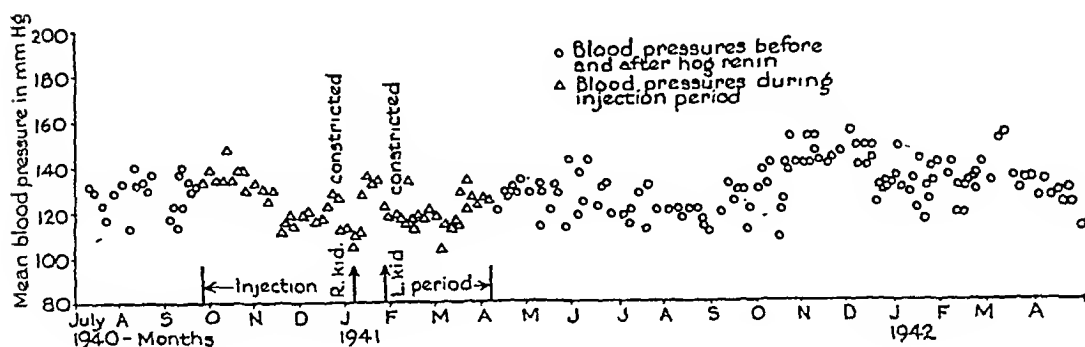
Fig. 1
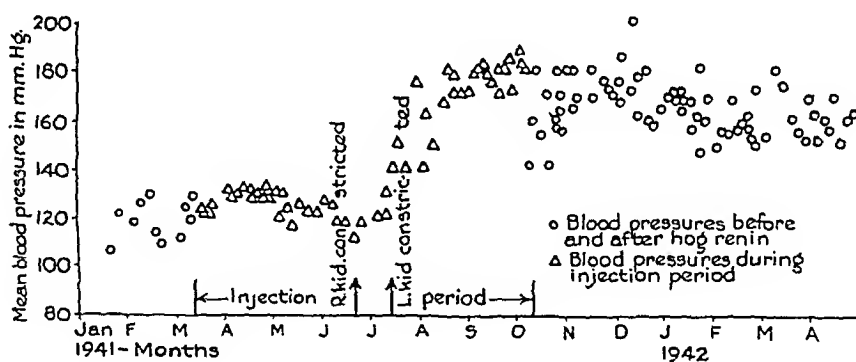
Fig. 2

Repeated assays for antirenin against both dog and hog renins gave negative results in all four dogs. No toxic effects from injections were detected in any of the animals.

Dog renin. There were no significant changes from the initial control levels in the blood pressures of this group of four dogs during the three months of injections of dog renin prior to the first renal artery constriction. Two of the dogs remained normotensive following bilateral renal artery constriction. One of these continued to be normotensive during the three months of continued dog renin treatment following constriction and during the five months of observation subsequent to dog renin injections. The other animal remained within normotensive limits during the period of dog renin injections following the arterial

constrictions but showed a gradual though significant rise in blood pressure during the five months succeeding dog renin injections. The two remaining dogs developed hypertension following arterial constriction. In one of these dogs hypertension appeared after the second renal artery was constricted; in the other unilateral constriction produced hypertension. The hypertensions of both animals continued essentially unchanged during three months of continued dog renin injections and five months of observation subsequent to the termination of the injections.

Antirenin assays against dog and hog renins were repeatedly negative in all four dogs. No toxic manifestations from the injections were observed.

Rabbit renin. The normotensions of the two dogs in this group were not altered from their control levels during the first three months of rabbit renin injections. Following unilateral constriction the pressures continued to be normotensive. Shortly after constriction of the second renal artery, one of the two dogs died of pneumonia. The other animal remained persistently normotensive not only during the three additional months of rabbit renin injections but also during the five months subsequent to rabbit renin treatment.

Antirenin, tested against rabbit, dog, and hog renins, appeared in the serums of the two dogs during the second month of injections and disappeared during the second month after the injections were discontinued in the surviving dog. Untoward effects from the injections were never observed in the dogs.

Inactive human renin. The normotensions of two dogs observed during the initial control period were unchanged by three months of inactive human renin injections prior to constriction of the renal arteries. Unilateral renal artery constriction produced hypertension in both animals which was accentuated somewhat by constriction of the opposite renal artery. Two weeks subsequent to the second constriction, one of the dogs died of malignant hypertension. The hypertension of the other animal persisted during the remaining three months of inactive human renin injections and during the one month which has elapsed since treatment was stopped.

Antirenin was never demonstrated in the serums of these two dogs and they never displayed any toxic effects from the injections.

Liver extract. The normotensions of the three dogs in this group did not show any significant changes during the pre-constriction injection period. Following constriction of the first renal artery, all three of the dogs developed hypertension which was intensified by constriction of the second renal artery. Two weeks subsequent to the second operation, one of the dogs died of malignant hypertension. The other two dogs have shown no significant changes in their hypertensions during the post-constriction injection period.

Repeated serum examinations never showed any antirenin and the extract produced no toxic effects.

Untreated controls. After two to four months of control blood pressure readings, sixteen dogs were subjected to bilateral renal artery constriction. Following constriction of the first renal artery, fourteen of the dogs became hypertensive. The remaining two developed hypertension after the opposite renal artery

was constricted three weeks later. These sixteen control dogs showed persistent hypertensions during the three to five months of observation following constriction. Antirenin was never present in the serums of the dogs. Their body weights, blood urea nitrogens, and urines remained essentially unchanged.

DISCUSSION. These experiments constitute the first successful prophylaxis of experimental renal hypertension in the dog. The results show that of a total of fifteen dogs treated with renal extracts, six (40 per cent) were protected against hypertension and nine were not. This is in striking contrast to the control group of sixteen untreated dogs all of which became hypertensive. In a larger series of 75 untreated dogs operated upon by essentially the same technique during the past three years, we have failed to obtain some degree of hypertension in only one animal. Another laboratory reports failure to obtain hypertension following constriction of the renal arteries by a somewhat different technique in 10 per cent of their dogs (6). Also pertinent to the prophylactic effect of certain of these renal extracts is the fact that whereas fourteen of the sixteen control dogs and 64 of the 75 dogs in the larger series developed hypertension following constriction of the first renal artery, only six of the fifteen renal extract treated dogs did so.

The mechanism of these prophylactic effects is not apparent at present. Obviously the small number of animals in each experimental group and the lack of consistent results within each group make a final interpretation impossible. Whether the prophylactic effects are due to *renin or to some other substance or substances in the partially purified solutions* is likewise not determinable at present. The evidence that the prophylactic effects are not due to antirenin appears conclusive. Thus two dogs given dog renin and one dog given inactivated hog renin were protected against experimental renal hypertension although these animals never at any time showed antirenin in their serums. Furthermore, the two dogs treated with hog renin and the dog injected with rabbit renin which were protected against hypertension continued to be normotensive for months following the disappearance of antirenin from their serums. Nevertheless, some other type of antihormone or immune response is not completely ruled out.

Before the question of mechanism can be answered we must conduct studies on larger groups of dogs treated prophylactically and therapeutically with various partially purified renin solutions. We must also study the effects of highly purified renins as well as the influence of dosage and of route of administration. Such studies are now under way.

The long periods of normotension in five of the protected dogs following the discontinuance of treatment are striking. We intend to observe these animals for a minimum of eighteen months subsequent to treatment. If these five dogs do not become hypertensive during the observation period of eighteen months, we shall further constrict the renal arteries until hypertension, malignant hypertension, or fatal uremia without hypertension results.

As stated, we observed a notable lack of toxic effects from the injections although the most sensitive tests for renal, hepatic, and other functions were not

employed. The toxic effects of partially purified renin solutions noted by Winternitz et al. (7) and Leiter and Eichelberger (8) are probably related chiefly to their employment of the intravenous route. All of our injections have been given intramuscularly. Moreover, the toxic substances demonstrated by Winternitz and Leiter in their partially purified kidney extracts are not necessarily in any way related to the prophylactically potent principle or principles in the partially purified renal extracts which we have employed.

SUMMARY

1. Studies were made of the prophylactic effects of partially purified hog renin, inactivated hog renin, dog renin, rabbit renin, inactive human renin, and liver extract prepared like renin, in experimental renal hypertension in the dog.

2. Hog renin completely protected two dogs, partially protected one, and did not protect a fourth animal against the development of experimental renal hypertension following constriction of the renal arteries by the Goldblatt technique.

3. Inactivated hog renin protected one dog but did not protect three other animals.

4. Dog renin completely protected one dog, partially protected one, and did not protect two dogs.

5. Rabbit renin completely protected one dog against experimental renal hypertension.

6. Inactive human renin offered no protection to two dogs and liver extract was likewise ineffective in three dogs.

7. Sixteen untreated control animals all developed experimental renal hypertension following constriction of the renal arteries.

8. The mechanism of these prophylactic effects is not apparent at present. They may be due to renin or to some other substance in the partially purified renal extracts. Antirenin is almost certainly not involved. Further studies which may clarify the mechanism are now under way.

REFERENCES

(1) WAKERLIN, G. E. AND C. A. JOHNSON. J. A. M. A. 117: 416, 1941.
(2) DAMESHEK, W. AND J. LOMAN. This Journal 101: 140, 1932.
(3) GOLDBLATT, H., J. LYNCH, R. F. HANZAL AND W. W. SUMMERVILLE. J. Exper. Med. 59: 347, 1934.
(4) GROSSMAN, E. B. Proc. Soc. Exper. Biol. and Med. 39: 40, 1938.
(5) JOHNSON, C. A. AND G. E. WAKERLIN. Proc. Soc. Exper. Biol. and Med. 44: 227, 1940.
(6) KATZ, L. N. Personal communication.
(7) WINTERNITZ, M. C., E. MYLON, L. L. WATERS AND R. KATZENSTEIN. Yale J. Biol. and Med. 12: 623, 1940.
(8) LEITER, L. AND L. EICHELBERGER. J. Mt. Sinai Hosp. 7: 744, 1942.

A STUDY ON SPERMATOGENESIS IN RATS

EUGENE CUTULY

From the Department of Anatomy, Wayne University Medical School, Detroit, Michigan

Adequate evidence has been adduced that androgens will maintain (1, 2, 3) and initiate (4, 5) spermatogenesis in the hypophysectomized rat. This observation has been difficult to reconcile with the finding that male hormone may cause damage to the seminiferous epithelium in normal-immature rats (6). Such deleterious effects in the young rat have been considered generally to be brought about indirectly through suppression of the output of gonadotropic hormone from the pituitary (6). This interpretation leaves some problems unexplained. For example, it is difficult to understand why male hormones in small doses are damaging to seminiferous epithelium, while large doses may show no injurious effects whatever. The data which follow may afford a solution of such paradoxical phenomena.

MATERIAL AND METHODS. Twenty-nine litters, comprising 94 rats, have been used. Ranging in age from 25 to 44 days at the outset, their ages at the end of the experiments varied according to length of treatment. The animals comprised two groups: experimental and control. Experimental animals were hypophysectomized, and immediately injected subcutaneously with one of the following substances[1]: testosterone propionate (TP), pregnant mare serum (PMS), or with hypophyseal synergist.[2] Injections were repeated daily until the day preceding autopsy. Daily doses were as follows: 1, testosterone propionate, 1 or 3 mgm. in 0.3 cc. peanut oil; 2, pregnant mare serum, 1 or 2 R.U. in 0.5 cc. solution of trisodium phosphate adjusted to pH 7.9; 3, hypophyseal synergist, 1 or 3 mgm. in 0.5 cc. solution of tri-sodium phosphate. Control animals, littermates of experimental rats, were of 3 kinds; untreated hypophysectomized, initial and normal. Initial control rats were killed at the beginning of individual experiments while normal and hypophysectomized control rats were sacrificed at the end of individual experiments. Initial control values for testicular weights were obtained by sacrificing one animal of a litter at the start of an experiment, or by removing one testis from an experimental rat at the time of hypophysectomy. In the latter instance the weight of each single testis obtained at biopsy and at autopsy was doubled in order to make possible the presentation of average data in table 1. Autopsies were performed one day after the final injection. Organs were weighed in the fresh state, fixed in Bouin's fluid, and embedded in paraffin. Sections were cut at 7 to 10 μ and stained with hematoxylin and eosin. At autopsy saline smears of the head and tail of epididymides

---

[1] The hormones used in this study were supplied by Dr. Erwin Schwenk, Schering Corp., Bloomfield, N. J.

[2] Synergist was prepared from sheep pituitaries. It was stated to be chiefly follicle-stimulating, with traces of luteinizing principle.

were examined for spermatozoa.   If sperm were present, their motility or lack of motility was noted, and such observations were correlated with microscopical findings.

RESULTS.   Attention will be directed chiefly to: 1, weights of testes; 2, histological appearance of tubules; 3, histological appearance of interstitial cells; 4, observations on epididymides.   Wherever necessary the condition of the accessory sexual organs will be discussed.   It is helpful to recall (7) that testicular size or weight largely reflects the extent of tubular development, since interstitial

TABLE 1

Effects in hypophysectomized rats of daily injections of 1–3 mgm. of testosterone propionate (TP), 1–2 r.u. of pregnant mare serum (PMS), or of 1–3 mgm. of hypophyseal synergist (SYN)

AGE AT OPERATION	NUMBER OF LITTERS	NUMBER OF RATS	TREATMENT		TESTES	
			Substance	Number of injections	Average weight at autopsy	Change from initial control
days					*gm.*	*gm.*
25–28	4	11	IC		0.30	
		1	HC		0.08	−0.22
		3	TP	20–21	0.24	−0.06
		4	PMS	21–23	0.87	0.57
		2	SYN	18–23	0.97	0.67
		1	NC		2.11	1.81
29–34	14	6	IC		0.67	
		1	HC		0.13	−0.54
		18	TP	20–63	0.57	−0.10
		3	PMS	21	0.96	0.29
		2	SYN	21	0.96	0.29
		5	NC		2.41	1.74
35–44	11	8	IC		1.24	
		1	HC		0.20	−1.04
		24	TP	9–72	1.17	−0.07
		13	NC		2.63	1.39

IC, Initial control; HC, Hypophysectomized control; NC, normal control.

tissue constitutes but a small percentage of the testis.   Weights of testes and other data are given in table 1.

1. *Animals hypophysectomized between days 25 and 28.*   Sperm were not formed in testes of animals injected with TP, and the tubules were markedly damaged (cf. figs. 1 and 7).   Both PMS and synergist brought about distinct tubular development and sperm formation.   No significant change in weight of testes took place after injections of TP, but PMS and synergist brought about marked increases in testicular weight over initial control levels (table 1).   Atrophy of interstitial cells was not prevented by TP.   Interstitial cells were markedly stimulated by PMS, as judged histologically and by the development of seminal

vesicles and prostate. Synergist caused only slight stimulation of interstitial cells. In the epididymides of most of the treated animals were seen large numbers of cells, which presumably had become desquamated from the seminiferous epithelium (cf. figs. 2 and 8). Included among these cells were leucocytes, giant cells and spermatocytes, most of the latter showing degenerative changes. Adrenal glands of all treated animals were atrophic.
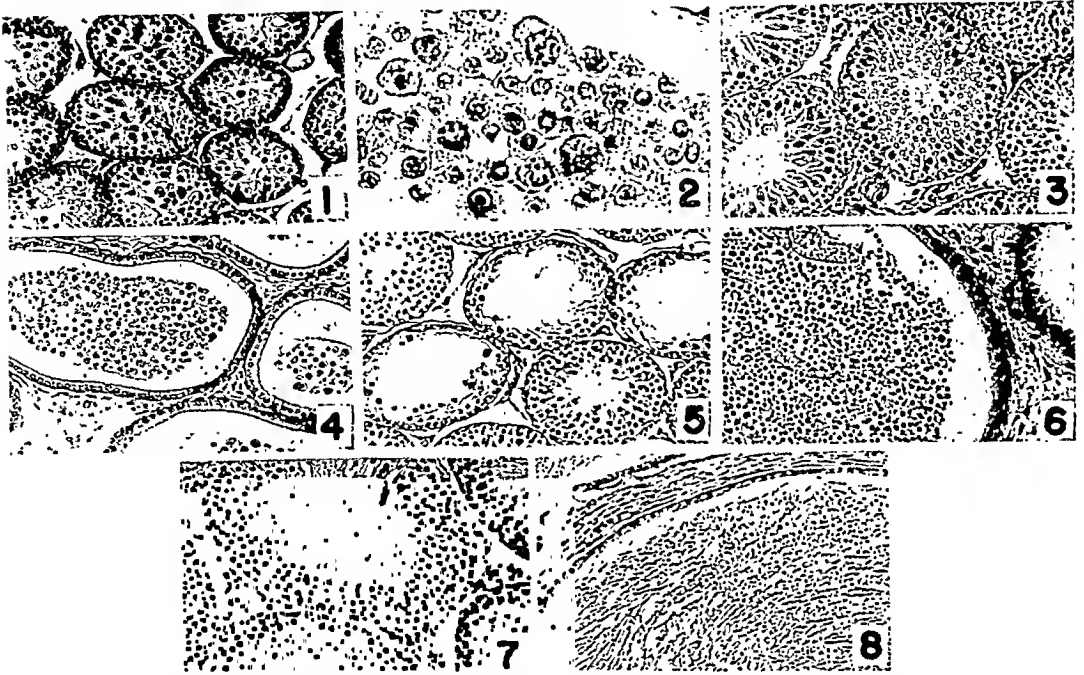
Fig. 1. Testis of rat hypophysectomized at age 25 days and injected daily for 21 days with 3 mgm. of testosterone propionate (TP). Atrophy of tubules and interstitial cells is marked.

Fig. 2. Epididymis of rat hypophysectomized at age 30 days and injected daily for 21 days with 3 mgm. of TP. Lumen contains gametes, some degenerating, desquamated from testis.

Fig. 3. Testis of rat described in figure 3. Sperm are visible in some tubules.

Fig. 4. Epididymis of rat hypophysectomized at age 32 days and injected daily for 71 days with 3 mgm. of TP. Germinal elements are present in the tubules.

Fig. 5. Testis of rat described in figure 4. Note tubules which have lost most of their germinal epithelium.

Fig. 6. Epididymis of rat hypophysectomized at age 35 days and injected daily for 42 days with 3 mgm. of TP. Clusters of spermatozoa may be seen among the mass of desquamated cells.

Fig. 7. Testis of normal control rat.

Fig. 8. Spermatozoa in epididymis of normal control rat.

2. *Animals hypophysectomized between days 29 and 34.* Spermatozoa were found in the testes of all treated rats, despite the average loss in testicular weight (table 1). In TP-treated animals sperm were not numerous. Tubules were often shrunken, although in some instances they were clothed with an epithelium similar in appearance (fig. 3) to that found in normal animals. Of

interest was the observation that some tubules which showed retrogressive changes frequently contained spermatozoa which, instead of being embedded in Sertoli cells, were situated haphazardly in the epithelium or lumen. Occasional non-motile sperm were observed in fresh smears of epididymides; few sperm were found in fixed preparations. Possibly some were missed in the masses of degenerating cells filling some of the tubules of the epididymides (fig. 4). These masses resembled those observed in the preceding group, and no doubt had sloughed off the germinal epithelium of the testes. Loss of germinal elements from the testicular tubules is well illustrated by figure 5. Interstitial cells were atrophic.

Tubular development was more uniform and more pronounced in animals treated with PMS and synergist than in animals which had received TP, as might have been expected on the basis of testicular weight increments. Sperm were more numerous both in the testes and epididymides. For the most part degenerating germ cells which had been transported from the seminiferous epithelium to the epididymis were not nearly so abundant as in animals which had been given TP. Interstitial cell stimulation was moderate following administration of PMS; after synergist, it was slight. Adrenal glands in all hypophysectomized animals were atrophic.

3. *Animals hypophysectomized between days 35 and 44.* Data in table 1 reveal that TP caused little change in average testicular weight. In spite of this apparently indifferent weight response, tubular development was of a higher order than in the younger groups. Some tubules were invested with a thick epithelium containing a normal complement of gametes in all stages of development. Other tubules were damaged to varying degrees, some containing only sustentacular cells.

Fresh smears of the epididymides and fixed preparations showed moderate numbers of spermatozoa. Some sperm in the heads, as well as in the tails, of the epididymides exhibited motility. Three animals from a litter hypophysectomized at age 37 days and injected with TP were, at intervals from the start to the end of the experiment, caged with estrous females of known fertility. Mating always took place, and vaginal smears often contained motile spermatozoa. In no instance, however, did pregnancy ensue. This would suggest a functional or numerical deficiency of spermatozoa. In addition to spermatozoa in the epididymides of TP-treated rats there were also masses of desquamated germinal elements (fig. 6). No gonadotropins were used in this series. Adrenals of all hypophysectomized rats were atrophic.

DISCUSSION. In the stock rats used for these experiments the earliest age at which spermatozoa appear in the testes is 34 days. If animals were hypophysectomized before this age, and injected with TP, the following results were noted: 1. No spermatozoa developed when the operations were performed before day 29, and tubules exhibited much damage. 2. Some spermatozoa formed when operations were performed on or after day 29. Many tubules, however, revealed degenerative changes. When hypophyseal or chorionic gonadotropin was administered to rats hypophysectomized between the ages of 25 to 34 days, sperm formed in all instances, the seminiferous tubules were uniformly stimu-

lated, and appreciable increases in weight over the preoperative levels occurred in most of the testes. If treatment with TP was instituted in rats hypophysectomized after sperm normally formed, spermatozoa developed in larger numbers and tubules showed fewer signs of retrogression than in the younger animals which received androgen.

Of particular interest was the occasional presence in the epididymides of masses of degenerating cells. These masses comprised giant cells, leucocytes, and various classes of gametes. They were found in the epididymides of nearly all hypophysectomized rats which received TP, and in the very young hypophysectomized animals which had been treated with gonadotropins. Few such cells were seen in the epididymides of older hypophysectomized rats injected with synergist or PMS.

The reason for the desquamation and transport of seminiferous epithelium into the epididymis is not known, although it is tempting to associate a high level of androgen with this phenomenon. Such correlation, of course, would be conjectural at this time. It seems worth noting, however, that in the normal immature male rat development of the prostate and seminal vesicles is a gradual process reaching a peak at 80 days of age (8). In normal development, at least, early spermatogenesis occurs in the presence of relatively small amounts of androgen.

On the basis of available data it does not seem reasonable to conclude that androgen is directly injurious to gametes, since many cells metamorphose into spermatozoa in the hypophysectomized (4, 9) or intact (10) rat. Rather it would appear that androgens in some way cause germ cells to become detached from the tubular epithelium and to be transported to the epididymis where they degenerate. Deleterious effects of androgenic substances on the spermatogenic epithelium in intact prepuberal rats (6) have been believed to be brought about indirectly by suppression of gonadotropic hormone in the anterior pituitary. Further work must be done to determine whether this latter theory is correct or whether the damage is occasioned by desquamation of gametes into the tubular lumina. The latter concept would serve to explain the apparently paradoxical observations (6) that the extent of testicular injury in immature intact rats treated with androgens is inversely proportional to the dose. It seems possible that the lower doses simply were inadequate to promote spermatogenic development, and that no mediation of the pituitary was involved in the reaction. The damage noticed in the testes of very young intact rats treated with androgen decreases with age (6, 10), and in hypophysectomized rats androgen causes increasingly better tubular development with age (present study, and 4, 9). Perhaps age itself is not so much a factor in this differential response. Possibly in very young rats androgen brings about desquamation of gametes so rapidly that restoration of a stratified epithelium by replacement from spermatogonia is impossible. This would account for the almost complete absence of germ cells which has been noted in some of the testes described in this report.

There is still no conclusive evidence that male hormones stimulate germ cells directly. If TP was gametokinetic in the present study, it differed in several respects from the gametokinetic gonadotropins. Both PMS and synergist

induced spermatogenesis in rats hypophysectomized before the age of 29 days; androgen failed in this respect. Sloughing of seminiferous epithelium was not so marked as to lead to tubular atrophy in rats receiving PMS or synergist; in TP-treated rats tubular desquamation was extensive.

This investigation throws little additional light upon the nature of the effect of androgen upon spermatogenesis. It does, however, emphasize that male hormone may directly induce sloughing of the germinal epithelium; and it suggests that transport of cells from the seminiferous tubules to the epididymis may be influenced by androgenic hormone.

SUMMARY

Testosterone propionate (TP), pregnant mare serum (PMS), and hypophyseal synergist were administered to rats hypophysectomized between the ages of 25 to 44 days.

1. Animals hypophysectomized before day 29. Both gonadotropins induced good tubular development and sperm formation. Testes of TP-treated animals showed much tubular damage and no differentiation of spermatozoa. Masses of degenerating cells, among which many gametes were recognized, were seen in the epididymides.

2. Animals hypophysectomized between days 29 and 34. Sperm developed in all treated animals. Both gonadotropic substances caused pronounced and uniform tubular growth and development. Testes of TP-injected rats exhibited considerable sloughing of seminiferous epithelium. Epididymides of rats receiving TP contained large numbers of degenerating elements transported from the testes.

3. Animals hypophysectomized between days 35 and 44. Only TP was used in this series. Although there was little change in testicular weight, as compared with preoperative levels, tubular development was more uniform than in preceding groups, and greater numbers of spermatozoa formed. Some tubular damage was noticed, and most of the epididymides contained elements which had become detached from the seminiferous epithelium.

Tubular damage following use of TP in intact and hypophysectomized prepuberal rats is discussed in the light of germ cell desquamation. The possibility that androgen influences transport of germinal cells from testis to epididymis is suggested.

REFERENCES

(1) WALSH, E. L., W. K. CUYLER AND D. R. McCULLAGH. This Journal 107: 508, 1934.
(2) CUTULY, E., D. R. McCULLAGH AND E. C. CUTULY. This Journal 119: 121, 1937.
(3) NELSON, W. O. Cold Spring Harbor Symp. Quant. Biol. 5: 123, 1937.
(4) CUTULY, E. AND E. C. CUTULY. Endocrinology 26: 503, 1940.
(5) NELSON, W. O. Anat. Rec. 79: 48 (suppl.), 1941.
(6) MOORE, C. R. AND D. PRICE. Endocrinology 21: 313, 1937.
(7) CUTULY, E. AND E. C. CUTULY. Endocrinology 22: 568, 1938.
(8) PRICE, D. Am. J. Anat. 60: 79, 1936.
(9) CUTULY, E., E. C. CUTULY AND D. R. McCULLAGH. Proc. Soc. Exper. Biol. and Med.,
           38: 818, 1938.
(10) MOORE, C. R. and D. PRICE. Anat. Rec. 71: 59, 1938.

QUANTITATIVE STUDIES ON MUSCLE AND NERVE REGENERATION IN THE RAT[1]

H. M. HINES, J. D. THOMSON AND B. LAZERE

From the Department of Physiology, State University of Iowa

The literature contains numerous accounts of investigations dealing with the histologic aspects of axone regeneration in peripheral nerves. However, there is a paucity of quantitative information pertaining to the extent and velocity of functional restoration in the affected neuromuscular structures. The importance of such information is apparent when attempts are made to evaluate the effects of experimental conditions upon the course of regeneration.

This report is concerned with the results of experiments on the gastrocnemius muscles and tibial nerves of the albino rat. This animal was selected for study because of its genetic homogeneity, short life span and the wealth of information concerning its nutritional requirements. Studies on the histologic aspects of regeneration of peripheral nerves in this species have been reported by Greenman (1). The work herein reported is chiefly concerned with quantitative measurements of muscle strength, mass and creatine concentration at various times during the course of degeneration and subsequent regeneration. In addition studies were made concerning the presence of fibrillary activity and acetylcholine sensitivity. Attention was also given to the rôles of age, sex and body weight in the velocity and extent of regeneration.

EXPERIMENTAL METHODS. A total of more than 200 animals of known age which had been reared on a standard diet were selected for study from a closely inbred stock. The operative procedures were carried out under light ether anesthesia. A standard lesion was made in the tibial nerve of one limb at the level of its junction with the peroneal. This was accomplished by crushing the exposed nerve with a coarse linen ligature against a metal rod. The nerve was released from its tie by cutting through the ligature down to the rod. The lesions were made at the junction of the tibial and peroneal branches of the sciatic nerve in order to standardize the length of the nerve trunk involved. The tissues of the unoperated contralateral limb served as controls. Crushing was preferred to cutting and suture because by crushing the nerve sheaths remain intact and thereby afford a better alignment for reinnervation. That crushing gives complete axone separation and paralysis of the gastrocnemius muscle is attested by the following observations: *1.* Electrical stimulation of the nerve above the lesion immediately after the crushing resulted in no muscular contraction. *2.* Electrical stimulation of the nerve below the lesion after allowing three days for degeneration resulted in no muscular contraction. *3.* The rates of muscle atrophy, loss of strength and decreases in creatine and glycogen con-

centration were essentially the same in the muscles which had their motor nerve supply crushed as in those with cut motor nerves.

Most of the studies were made 12, 18, 21, 28, 35, 42, 56 and 84 days after denervation. Muscle strength was determined by measuring the maximal isometric tension that would develop upon direct and indirect stimulation. The tendon of Achilles was cut and attached to a Blix type torsion rod. A portion of the femur was exposed and fixed in a rigid clamp. The intact muscle was directly stimulated through two needle electrodes which pierced it, one at the tendon and the other at the origin. Adjustable silver electrodes were placed in contact with the tibial nerve for indirect stimulation. Short volleys of slightly supermaximal stimuli, either from an inductorium or as condenser discharges, were delivered to the muscle and nerve. The frequency and strength of the stimuli were such as were found to be adequate to give maximal tetanus tension. The extent of muscle shortening was measured from optical records. Muscle strength was considered to be the maximal tension developed in response to either direct stimulation of the muscle or of its motor nerve. Under the above experimental conditions the tension developed by normal muscle in response to nerve stimulation was approximately the same as that elicited by direct activation. At the conclusion of the strength measurements, the gastrocnemii were carefully dissected, weighed and analyzed for creatine.

Observations were made in a series of animals concerning the time of onset and disappearance of fibrillary activity. This was done by the amplification of action potentials led from needle electrodes buried in the intact muscle and from visual observation of the exposed muscles. The sensitivity of the affected muscles to intravenous injections of acetylcholine was determined in a number of animals. Such muscles were considered relatively insensitive to acetylcholine if the intravenous injection of 0.5 mgm. of acetylcholine failed to elicit a detectable contracture response in the muscles of animals which had previously received injections of 0.1 mgm. atropine sulfate and 0.1 mgm. eserine sulfate.

RESULTS. For a period of 12 days after denervation the muscles underwent atrophy at a rate comparable to that found in muscles with sectioned nerves when reinnervation was excluded. The rate of weight loss was much slower between the twelfth and twenty-first days (fig. 1). The continuance of some measure of weight loss after the onset of initial reinnervation is attributed to the fact that some muscle fibers made functional contacts with regenerating nerves sooner than others. Thus it appears as if for a certain period of time some fibers were undergoing regeneration while others were undergoing further atrophy.

The average values for the regeneration of muscle mass at 21 days and later times after denervation are given in table 2. The relationship of the rate of muscle regeneration to time for these periods has been found to be defined by the equation:

$$k = t \log_{10} \frac{W_{cp}}{W_r - w}$$

where k is a constant calculated from individual data, t is the time in days after denervation, W_{cp} is the relative mass of muscle cell phase in the normal contralateral control muscle (85 per cent of the weight of the muscle), W_r represents the relative mass of the regenerating muscle at time t and w equals the mass of non-muscle phase in normal muscle (15 per cent of the weight of normal muscle). The correlation coefficient for $1/t$ and $\log_{10}(W_r - w)$ was found to be 0.85. It is interesting to note that the formula for regeneration resembles that found to fit the growth rates of albino rats (3).

The total tension developed in response to direct muscle stimulation had decreased to 40 per cent of that in the contralateral control at the end of 12 days (fig. 1). During this time the loss of strength was found to be precisely that lost by muscles with severed nerves. After the onset of reinnervation the
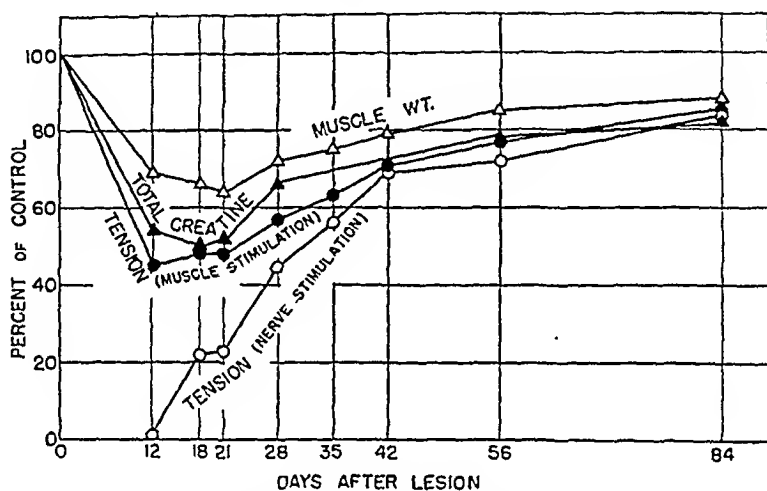
Fig. 1. A graph of the average values for the gastrocnemii at various times after denervation. The gastrocnemius muscle of the unoperated contralateral limb constituted the control.

muscles recovered strength. The recovery was due in part to an increased capacity of the muscle fiber per unit of mass to develop tension and in a larger measure to an increase in the size of the muscle with regeneration.

The creatine concentration of muscle (table 1) for the first twelve days following nerve crushing decreased by an amount comparable to that noted in muscle following nerve section (4). The restoration of muscle creatine is attributed to two factors, one related to an actual increase in concentration within the muscle cell, the other to an increase in the ratio of creatine rich muscle cell phase to creatine poor interstitial tissue. The latter factor appears to be chiefly responsible for the concentration changes taking place after 28 days.

Muscular contraction could not be elicited through motor nerve stimulation prior to 12 days after denervation. The first detectable signs of reinnervation usually appeared at 12 to 15 days after nerve crushing. Satisfactory measurements of the tension developed by muscle in response to nerve stimulation could be made after the eighteenth day. The tension values increased rapidly until

about the forty-second day and, thereafter, tended to parallel the rate of muscle regeneration (fig. 1).

Fibrillary contractions made their appearance at 48 to 72 hours after nerve crushing and persisted until about the sixteenth to eighteenth day. The time of their disappearance was approximately that for the earliest signs of reinnervation as measured by the ability of the nerve to activate its muscle. The denervated muscle displayed an increased sensitivity to acetylcholine. Following

TABLE 1

A summary of average values for control and experimental gastrocnemii

TIME AFTER LESION	NUMBER OF ANIMALS	TENSION PER GRAM MUSCLE WHEN ACTIVATED THROUGH				CREATINE IN MGM. PER 100 GMS. MUSCLE	
		Nerve		Muscle			
		Crushed	Control	Crushed	Control	Crushed	Control
days		*gms.*	*gms.*	*gms.*	*gms.*		
12	14			1204	1877	340	452
18	14	657	1954	1506	2074	353	450
21	33	728	1762	1469	1874	373	463
28	34	928	1421	1364	1715	410	458
35	20	1311	1571	1684	1908	396	451
42	9	1580	1820	1707	1877		
56	19	1442	1686	1629	1825	425	461
84	12	1502	1693	1538	1701	454	483

TABLE 2

The rate of muscle weight regeneration

TIME AFTER DENERVATION, t	NUMBER OF ANIMALS	RELATIVE WEIGHT OF REGENERATING MUSCLE,* W_r	$k t$
days			
21	34	61.9	5.47 ± 0.17
28	13	72.4	4.83 ± 0.38
35	20	73.0	5.89 ± 0.40
42	9	79.0	5.24 ± 0.51
56	19	85.2	4.73 ± 0.36
84	21	88.1	5.72 ± 1.13

* In per cent of that in contralateral control.
† The values for k and standard errors are calculated from individual data.

the onset of reinnervation this sensitivity when measured by the typical contracture response was found to decrease but some evidence of this state was still present as late as 35 days after nerve crushing. It is to be noted that the regenerating muscle exhibits an increased sensitivity to acetylcholine for a considerable period of time after the disappearance of fibrillary activity.

The results of experiments (fig. 2) in which determinations of muscle weight and strength were made 28 days after denervation in groups of animals 60, 96, 117, 180, 280, 292, 362 days of age at the time of operation indicate the influence

of age upon the rate of neuromuscular regeneration.   The data indicate that regeneration was slower in the older than in the younger animals and in certain age ranges (e.g., 96 to 117 days) a difference of only 21 days was sufficient to affect appreciably the regeneration rates.   It is believed that these effects were due in part to the rôle of age per se in regeneration rates and in part to the greater distance required for nerve growth in the older and larger animals.   The importance of the distance factor is emphasized by the results of experiments in which the standard lesion was made in the nerve of one limb at the junction of the peroneal and tibial nerves and in the contralateral limb as close to the muscle as possible. This allowed for an average difference of 17 mm. in the lengths of the crushed nerves.   Studies made 28 days after the operation showed that the muscles with distal lesions were more than 20 per cent heavier and stronger than those with the lesions at the higher levels.   It is recognized that appreciable growth occurred
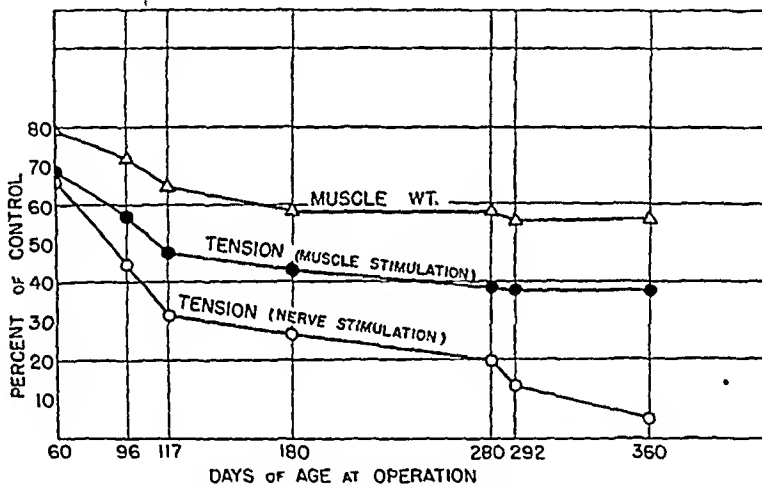
Fig. 2. Average values for the determinations made 28 days after denervation on animals of different ages.

in the muscles of the younger animals during the four weeks after operation.   It should be pointed out, however, that if corrections were to be made for any unequal growth increments in the control and experimental muscles they would tend only to emphasize further the effects of age upon the velocity of regeneration. The slightly faster rates of regeneration in the female groups are of the order of that required by the slightly shorter length of nerve pathway in the smaller females as compared with that in larger males of the same age.

Our results indicate that the extent and rate of muscle and nerve regeneration is remarkably constant in any group of rats of the same age, sex and body weight if attention be given to an exact location of the lesion.   Whenever possible the contralateral limbs should be employed as controls for the regeneration experiments, i.e., lesions placed in the nerves of the two limbs, one serving as the experimental member, the opposite as its control.   However, where this is impossible, as in drug and nutrition studies, it is necessary to employ animals

paired as to age, sex and body weight in order to allow a proper evaluation of any regeneration studies.

SUMMARY

A study has been made of regeneration in the gastrocnemius muscles and tibial nerves of more than 200 albino rats at 12, 18, 21, 28, 35, 42, 56 and 84 days after denervation.

Regeneration after nerve crushing was remarkably constant in animals of the same age, sex and body weight. It was definitely slower in older than in younger animals.

The earliest signs of functional reinnervation appeared at 12 to 15 days after denervation. At approximately this time fibrillary activity had disappeared but an increased sensitivity to acetylcholine was noted for as long as 35 days.

Muscle strength as measured by isometric tension responses to direct and indirect (nerve) stimulation increased rapidly after initial reinnervation until the thirty-fifth day. Subsequent recovery of strength was slower and was largely accounted for by the increase in muscle mass.

The data for the rate of regeneration of muscle weight was found to fit a formula essentially that expressing the body growth rate.

At 84 days the muscles undergoing regeneration possessed from 80 to 90 per cent of the creatine content, weight and strength of their contralateral controls.

REFERENCES

(1) GREENMAN, M. J. J. Comp. Neurol. 23: 479, 1913.
(2) KNOWLTON, G. C. AND H. M. HINES. Proc. Soc. Exper. Biol. and Med. 35: 394, 1936.
(3) ZUCKER, T. F., L. HALL, M. YOUNG AND L. ZUCKER. J. Nutrition 22: 123, 1941.
(4) HINES, H. M. AND G. C. KNOWLTON. This Journal 104: 379, 1933.

THE EFFECT OF ADRENALIN ON THE OXYGEN CONSUMPTION OF THE FISH, GIRELLA NIGRICANS

DIETRICH C. SMITH AND SAMUEL A. MATTHEWS

From the Department of Physiology, School of Medicine, University of Maryland, Baltimore, and Department of Biology, Williams College, Williamstown, Massachusetts

Studies on the respiratory metabolism of fishes have shown even prolonged treatment with thyroid substance fails to produce an increase in oxygen consumption (Etkin, Root and Mofshin, 1940). In this respect the metabolism of fishes differs markedly from that of mammals. As to the effect of adrenalin on the oxygen consumption of fishes, we are not aware of any studies on this point. To ascertain what such an effect might be we undertook the following investigations, the results of which indicate that fishes respond to adrenalin, as they do to thyroxine, in a manner quite different from that typically seen in mammals. These studies were made at the Scripps Oceanographic Institute of the University of California at La Jolla. We are greatly indebted to Dr. F. B. Sumner and to Dr. H. U. Sverdrup and the other members of the staff for the many courtesies extended to us while carrying on this work.

METHODS. Because of its availability and size, Girella nigricans, a teleost fish common to the shore waters of the California coast, was chosen for this work. From stock animals well adapted to aquarium conditions fifty to ninety gram specimens were selected and carefully weighed before the experiments were begun.

Oxygen consumption was determined by an open circuit method. The apparatus consisted of a set of wide-mouthed bottles, with a capacity of about two liters, in which the animals fitted comfortably. While the fish were restless during the first few hours in the containers, ultimately they quieted down and remained practically motionless for protracted periods. Their stay in the containers varied from three to seventeen days. Experience taught us that feeding was necessary if we wished to maintain them in good health for more than five or six days. In two instances experiments were done on the day after feeding and in neither case were the results significantly different from those obtained on animals that had fasted for twenty-four hours or more.

The respiration chamber itself was placed in a bath the temperature of which was thermostatically maintained between 19.5°C. and 20.5°C. Sea water was circulated through the chamber at a fixed rate which was determined daily. The rate of flow from chamber to chamber varied from 100 to 200 cc./min. and when properly adjusted this rate did not vary more than 2 cc. during the course of the day, providing the chamber was not disturbed. Special precautions were taken to prevent bubbles from forming in the bottles. When an occasional bubble did form the chamber was opened, the bubble removed and a day allowed for equilibrium to be reestablished. Accumulated organic material such as

feces, which appeared only after feeding or immediately after placing the fish in the bottle, was treated in the same way.

Samples of sea water were taken before and after having passed through the chamber. Special flasks suitably designed for this purpose were used and the usual precautions taken to prevent contamination with air. Oxygen determinations were made with the Winkler method. Duplicate samples were taken and each sample was analyzed separately. From the differences in oxygen content of the inflow and outflow waters, and from the rate of flow through the chamber, the amount of oxygen removed by the fish was readily calculated. This was expressed in cc./gm./hr. No attempt was made to determine carbon dioxide production nor to calculate the respiratory quotient.

Conceivably a certain fraction of the oxygen difference between inflow and outflow waters might be due to oxygen utilization by bacteria and plankton

TABLE 1

Oxygen consumption of girella nigricans in cc./gm./hr.

FISH NO.	WEIGHT	DAYS IN RESPIRATION CHAMBER																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	grams																			
1A	61.5	0.143		0.110	0.137	0.120	0.105*	0.118	0.094	0.123		0.113	0.068							
1B	81.0		0.110	0.099	*	0.167	0.165	0.126	0.111	0.113	0.123									
2A	51.2	0.296	0.129	0.099	0.106	0.106	0.086	0.129	0.131	0.197	0.142		0.095							
2B	68.9	0.146		*	0.199	0.135		0.159												
3A	52.9	0.171	0.170	0.125	0.145	0.258	0.181	*	0.163	0.147	0.148	0.098		0.063	0.119	0.100	0.120	0.103		*
3B	78.5	0.131		0.111																
4A	75.0				0.142	0.088		0.109	0.107											
4B	56.5		0.220	0.140		*		0.218	0.203	0.244										
5A	59.0		0.116		0.119	0.096		0.102	0.104											
5B	78.5	0.105	0.109	0.112		0.110		0.079	0.084											
6A	89.0		0.077	0.123	0.125	0.127		0.119	0.098	0.149	0.105	0.098								
6B	90.9	*	0.141	0.130		0.127														

* Fed.

present in the sea water flowing through the chambers. Checks were therefore made on the oxygen content of the water before and after flowing through a chamber containing no fish. In no case was any significant difference obtained. Therefore at the rate of flow used the oxygen consumption of any micro-organisms present in the water was so slight as to be of little or no consequence and was therefore ignored in calculating the results.

What might be called a resting or basal oxygen consumption was determined on each fish every morning whenever possible. This was not done if any bubbles had accumulated in the chamber during the night. Records of these determinations are presented in table 1. They show the length of time required for each fish to adapt itself to the bottles and the daily fluctuations in their oxygen consumptions under the conditions of the experiment. Since the samples were always collected in the morning after an all night period of equilibration and before the animals were disturbed in any way by laboratory procedures the values are probably as near basal as it is possible to get with this method. These results

were used as control values for any experiment done upon the fish during that day.

RESULTS. To know what effect adrenalin might have on oxygen consumption it was necessary to determine first how this might be changed by handling the fish preparatory to injection. Therefore a series of control experiments was performed in which 0.5 to 1.0 cc. of sea water was injected intraperitoneally in the same manner and amount as was adrenalin. The fish were immediately returned to the bottles after the injection and samples were collected from time to time. Since chloretone was present as a preservative in concentrations of 0.5 per cent in the adrenalin used it was also necessary to determine what effect if any this substance might have on oxygen consumption. To this end 0.2 cc. to 0.3 cc. of 0.5 per cent chloretone was injected in the fish, amounts equivalent to that introduced on the injection of adrenalin. Table 2 summarizes the data

TABLE 2

Oxygen consumption in girella nigricans after control injections of sea water or 0.5% chloretone

Oxygen consumption in cc./gm./hr.

FISH NO.	WEIGHT		BEFORE INJECTION	MINUTES AFTER INJECTION												
				30	45	60	75	90	105	120	135	150	165	180	270	300
	grams															
1B	81.0	0.5 cc. sea water	0.110			0.159									0.098	
1B	81.0	0.3 cc. chloretone	0.099			0.167										0.103
3A	52.9	1 cc. sea water	0.119			0.157									0.112	
3A	52.9	0.2 cc. chloretone	0.103	0.098		0.124		0.125		0.102		0.099	Dead	next	morning
3B	78.5	0.3 cc. chloretone	0.131		0.174		0.178		0.147		0.134				
4B	56.5	0.3 cc. chloretone	0.140		0.210										
4B	56.5	0.2 cc. chloretone	0.218	0.208		0.266		0.256		0.244					
5B	78.5	0.5 cc. sea water	0.105					0.078							
5B	78.5	0.3 cc. chloretone	0.112		0.181		0.154		0.144		0.126		0.103		
6A	89.0	1 cc. sea water	0.098			0.166										0.126
6B	89.0	0.3 cc. chloretone	0.105		0.115										

obtained from these control experiments. As the table shows the injection of either sea water or chloretone appreciably raised the oxygen consumption during the first hour following injection. Following this the oxygen uptake either began to fall immediately or tended to remain at the higher level for about a half an hour longer, in both cases reaching the pre-injection level some two or three hours later. Since the effects of injecting sea water and chloretone were found to be the same, it was concluded that these concentrations of chloretone had no effect on oxygen utilization in these fishes.

The results indicate that increased muscular activity of the fish aroused by handling was sufficient to produce a marked increase in oxygen consumption. It must be emphasized here that the method used does not precisely follow the changes in oxygen utilization. When for any reason the oxygen requirements of the fish change it takes some time for the chamber to establish a new equilibrium between this new value and the flow of water through the chamber. It is doubt-

ful whether this method ever exactly gives the true maximum or minimum unless this is maintained for some time, but it does very definitely indicate the direction of the change and gives an approximately correct idea of its magnitude.

To determine the effect of adrenalin upon oxygen consumption amounts varying from 1.0 cc. of 1/1000 to 0.2 cc. of 1/10000 adrenalin chloride were injected intra-peritoneally, as in the case of sea water and chloretone. After injection

TABLE 3

Effect of adrenalin upon oxygen consumption of girella nigricans
Oxygen consumption in cc./gm./hr.

FISH NO.	WEIGHT	ADRENALIN INJECTION	BEFORE INJECTION	MINUTES AFTER INJECTION												REMARKS
				30	45	60	75	90	105	120	135	150	165	225	240	
	grams	cc.														
1B	81.0	0.2 1/1000	0.113		0.185		0.189		0.150							—*
5B	78.5	0.5 1/10000	0.110	0.121			0.141		0.126		0.114					—
5B	78.5	0.2 1/10000	0.084		0.149		0.148		0.145							—
6B	91.0	0.2 1/1000	0.127				0.124		0.138		0.134					—
6B	91.0	0.3 1/10000	0.130		0.153		0.145		0.141		0.128		0.112			—
1B	81.0	0.2 1/1000	0.167	0.122		0.163		0.180		0.162		0.145				Pale 24 hrs. after food
1B	81.0	0.3 1/1000	0.126	0.045		0.050		0.050		0.058						Splotchy
2B	68.9	0.1 1/1000	0.199	0.168		0.176		0.201		0.171		0.177				Pale 24 hrs. after food
3A	52.9	0.5 1/1000	0.100				0.040								0.109	Comatose at 75', later revived
3B	78.5	0.2 1/1000	0.111		0.056		0.053		0.059		0.114					Splotchy
4B	56.5	0.2 1/1000	0.203		0.086		0.096		0.143		0.160		0.175			Splotchy
5B	78.5	0.2 1/1000	0.079		0.053		0.048		0.060		0.074		0.091			Splotchy
6A	89.0	0.5 1/1000	0.149			0.144									0.075	Pale
6B	91.0	0.2 1/1000	0.141	0.066		0.104		0.122			0.119					Pale
2A	51.2	0.5 1/1000	0.094					0.044								Died
2B	68.9	0.2 1/10000	0.158		0.079		0.073		0.050		0.043					Died, gall bladder punctured on injection
4A	75.0	1.0 1/1000	0.117			0.083										Died
4B	56.5	0.2 1/1000	0.244		0.049											Died
5A	59.0	1.0 1/1000	0.104			0.122										Died
6A	89.0	0.3 1/1000	0.098		0.033		0.038		0.040		0.044		0.038	0.017		Died

* — = No color change.

the fish were replaced in the bottles and samples were taken at varying intervals and analyzed. The results may be divided into three groups as shown in table 3.

In the first group were fish which received the smaller doses of adrenalin. The fish falling into this category showed a change in their oxygen consumption which paralleled that seen in animals injected with sea water or chloretone. These fish also showed no color change on injection. As is well known adrenalin in adequate amounts produces a marked paling of teleost fishes due to a concentration of all pigment within the melanophores. This paling occurs quite

independently of the color of the background or any other environmental factor. The presence or absence of such a color response tells whether adrenalin is present in the blood in sufficiently high concentration to affect the pigment cells. Although there is no proof that the dose of adrenalin capable of affecting these cells is the same as that affecting the oxygen utilization of the animal, we may reasonably expect it to be at least of the same order of magnitude. For this reason, together with the close resemblance to the responses seen in the control animals, it was concluded that the injected adrenalin had no effect on the oxygen consumption of these fishes because the amounts given were too small.

The second group comprised those fish which either paled completely on injection or else showed pale splotches on their sides. The latter are designated as "splotchy" in table 3. All these fishes without exception showed a marked fall in oxygen consumption, maximal about thirty minutes after injection. The pre-injection level was not attained until two or three hours later. This fall in oxygen consumption produced by the injection of adrenalin is all the more striking since, as has been shown, the injection of control animals always occasioned a definite rise. No attempt was made to relate the amount of fall to the size of the dose. We felt such an attempt would be futile for in spite of all reasonable precautions we could never be sure how much of the injected dose actually stayed in the animal. On withdrawal of the needle a certain amount of adrenalin often oozed out of the puncture in the fish's side. Animals in this group showed no outward change other than paling. This paling disappeared and was replaced by the original dark color about two hours after injection, coincident with the return of oxygen consumption to pre-injection levels. This confirms our view that the two responses have approximately the same threshold.

The third group, the animals receiving the largest doses, behaved in still a different manner. As was to be expected, they all showed marked paling shortly after injection accompanied by a marked fall in oxygen consumption. This fall, instead of reversing itself within an hour or so, continued until the animal died. Death occurred anywhere from two to three hours after injection. Obviously the dose of adrenalin injected was toxic. During the entire time up to and after death the animal remained pale.

Discussion. Adrenalin, then, in doses ranging from 1 cc. of 1/1000 to 0.2 cc. of 1/10000 either depresses oxygen consumption in Girella or affects it not at all. The largest doses in this range are toxic and the smallest are ineffective. The intermediate doses depress oxygen consumption without visibly affecting the behavior of the fish. The margin between lethal and non-lethal doses appears to be narrow. This raises the question as to whether the observed response to adrenalin can have any physiological significance. We are not prepared at the moment to answer this with any finality.

The parallelism between the effective depressing dose on oxygen consumption and the dose capable of concentrating the melanophore pigment is, however, striking and deserves emphasis. Whether adrenalin plays any physiological rôle in regulating color change is a controversial point. Certain fishes are known to pale when excited. In fact, the term "excitement pallor" is a common

onc in the literature on color changes. But this does not necessarily mean that excitement leads to the release of adrenalin, although the weight of the evidence strongly favors this view. If adrenalin is responsible in fishes for "excitement pallor" it might also at the same time depress oxygen consumption. It would be interesting to know if adrenalin originating in the fish's own body is capable of doing this in the face of increased muscular activity, as is injected mammalian adrenalin. As a rule "excitement pallor" in fishes is a transitory phenomenon and lasts but a few minutes.

The dose of adrenalin required to produce either a change in color or a change in oxygen consumption in Girella is huge as compared to doses capable of producing responses in mammals. The adrenalin used in these experiments was of course derived from mammalian glands. Whether adrenalin derived from fish chromaffin tissue (if such is possible) would have the same threshold as mammalian adrenalin is doubtful. It is difficult to believe that the adrenalin secreting tissue of the fish would be capable of secreting such large amounts in such a short time.

In the mammal adrenalin produces a rise in oxygen consumption of considerable magnitude and its release is generally held to be one of the mechanisms responsible for quickly increasing heat production. The fish, of course, is not confronted with the problem of regulating body temperature. Recently Barker, Fazikas and Himwich (1936) have shown that while adrenalin injected subcutaneously in the rat will markedly increase oxygen consumption, in the thyroidectomized animal similar doses produce a 20 to 25 per cent fall. No attempt was made to determine the degree of activity of the thyroid of the fishes used in our experiments. From what is known of other teleosts it is possible that this gland was physiologically inactive, a condition which might have some bearing on our results.

SUMMARY

Adrenalin when given intra-peritoneally in effective doses depresses the oxygen consumption of the fish, Girella nigricans.

REFERENCES

BARKER, S. B., J. F. FAZIKAS AND H. E. HIMWICH. This Journal 115: 415, 1936.
ETKIN, W., R. W. ROOT AND B. P. MOFSHIN. Physiol. Zool. 13: 415, 1940.

THE USE OF RADIOACTIVE PHOSPHORUS FOR DETERMINING CIRCULATING ERYTHROCYTE VOLUMES

RUBERT S. ANDERSON

From the Memorial Hospital, New York, N. Y.

Several methods, essentially the same in principle, have been used for determining the circulating erythrocyte and, especially, plasma volumes in animals.

Radioactive elements appear to have two advantages for such work. Their use need introduce no foreign compound and their determination is completely specific in any blood into which other radioactive elements have not been introduced.

Radioactive iron combined in the hemoglobin of the erythrocyte (1, 2) has recently been used for determining erythrocyte volumes. Iron has the great advantage of essentially permanent retention within the cell, but requires a donor animal to furnish the cells. Hahn and Hevesy (3) have used radioactive phosphorus. Their method also specifies a donor animal to furnish cells containing the radioactive phosphorus as organic phosphate.

Since phosphate containing the radioactive P^{32} was available through the courtesy of Dr. John H. Lawrence, experiments were started with it but along simpler lines. Phosphate leaves circulating plasma very rapidly but it seemed possible that phosphate introduced into the erythrocytes in vitro would, after reintroducing the cells into the circulation, be retained long enough to be of value. That ordinary phosphate enters the erythrocyte and that it does so with a large temperature coefficient has been shown by a number of authors, most recently by Halpern (4) who also gives references to the earlier work. The large temperature coefficient indicates that the process is more involved than simple diffusion. This work has been confirmed with radioactive phosphate on rabbit (5, 6) and human (7) erythrocytes. At 37°C. Aten and Hevesy report that 50 per cent of the radioactive phosphate entered rabbit erythrocytes in 3 hours while 22 to 44 per cent entered human erythrocytes in 4 hours.

In the work here reported the net rates of transfer of radioactive phosphate into and out of rabbit erythrocytes in vitro were studied first. The rate of loss was low enough so that similar experiments in vivo with cells containing radioactive phosphate were worth trying. Data have therefore been obtained on the rate of loss of radioactivity from the circulating blood of rabbits into which such cells had been introduced. From these same data values for the circulating erythrocyte volumes and the apparent circulating blood volumes have also been calculated.

METHODS. For the in vitro experiments disodium hydrogen phosphate, 0.2 to 0.4 mgm. per ml., containing P^{32} was added to rabbit blood and a gas mixture containing 5 per cent carbon dioxide and 95 per cent oxygen or nitrogen was

bubbled through it. The blood was then incubated at 37°C. with stirring by hand every 15 minutes and 0.1 or 0.2 ml. samples were withdrawn at various times. The determination of the radioactivity[1] of the washed cells and of the plasma plus washings was done, after drying at 100°C., on a Geiger-Müller counter.

The rate of loss of radioactive phosphate from erythrocytes was determined on cells incubated under the conditions given above, washed with sodium chloride solution and resuspended in fresh plasma containing no active phosphate. The suspension of cells was then incubated as before and samples withdrawn at intervals. The cells of the samples were again washed and the radioactivity of the cells and of the plasma plus washings determined.

White rabbits, three to five months old, were used for the experiments. A 2 or 3 ml. sample of blood was drawn for each experiment, with 0.1 to 0.2 per cent potassium oxalate as an anticoagulant, and the cells allowed to pick up radioactive phosphate as described above. After about 3 hours, the cells were centrifuged, washed, suspended in sodium chloride solution and a carefully measured volume (2–3 mls.) of the cell suspension was reinjected intravenously into the ear of the same rabbit from which the cells had been drawn. Samples of blood were then obtained from the opposite marginal ear vein at various times after injection to see how completely the radioactivity was retained in the circulation. These samples were collected in 0.2 ml. pipettes containing anticoagulant. The radioactivity was determined after the sample had been dried.

The percentage of cells in the blood of each rabbit was determined after centrifuging the blood 20 minutes and 30 minutes at 4000 r.p.m. in 0.7 to 0.8 mm. capillary tubes. The average values obtained in each of two complete experiments done one week apart were 35.7 per cent (33.2–38.6) and 36.3 per cent (34–38.9) cells.

RESULTS. The two lower curves in figure 1 show the maximum and minimum rates of entrance of retained radioactive phosphate in the last dozen experiments. The vertical coördinate gives the fraction of the total radioactive phosphate added which remained in the cells after washing.

The upper curves in figure 1 show representative rates of loss from cells. It is evident that the rate of loss in vitro is much slower than the rate of entrance under these conditions. Apart from old cells which gave anomalous results, only one experiment showed a rapid rate of loss. The slower rate of loss as compared to entry under these conditions is not especially surprising. It seems reasonable, for instance, that the gradient of inorganic phosphate containing P^{32} may be greater going into the cell because of reactions inside which maintain the radioactive inorganic phosphate at a low concentration there. Aten and Hevesy (5, 6) state that phosphate exchange in the more easily hydrolyzable portion (about one-half) of the ester phosphate fraction within the cell takes place very rapidly.

Figure 2 shows the rate of loss of activity from the circulation in nine experi-

ments with rabbits. The activity is given in arbitrary units, the average of the samples withdrawn within 25 minutes having been set equal to 100 for each experiment. Each individual value was then calculated relative to this. The curve as drawn represents the maximum rate of loss from the circulation and hence the least favorable experiments. This loss was about 8 per cent in the 20 minutes after the first sample. Each type of symbol represents a different experiment and it is clear that several experiments showed a definitely slower rate of loss than this.[2] Since, at least in normal animals, samples may be drawn safely ten minutes after injection, this rate of loss is not too great for determinations of circulating erythrocyte volume. Hahn and Hevesy (3) found a 6 per cent loss in 26 minutes from the soluble phosphate fraction and a 10 per cent loss in 60 minutes from the phosphatide fraction.

From the same set of experiments the total circulating erythrocyte volume can be calculated. This volume in milliliters equals the total radioactivity in the injected cells (microcuries) divided by the concentration of radioactivity
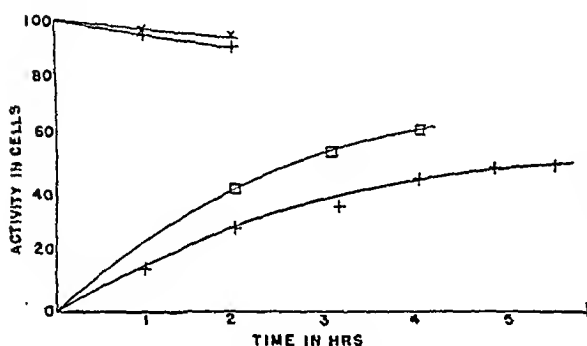
Fig. 1
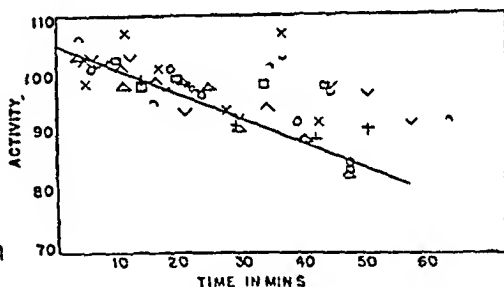
Fig. 2

(microcuries/ml) in the cells of the blood samples taken after the injection. This concentration was taken as equal to the concentration in the whole blood samples (microcuries/ml) divided by the average hematocrit value (36 per cent). The average concentration in the blood samples drawn within twenty-five minutes after injection was used for the calculation. Table 1 gives the data from seven experiments after dividing by the weight of the animal and reducing each value by 5 per cent to allow for extrapolation back to zero time.

From the circulating erythrocyte volume, or directly from the original data, an apparent circulating blood volume can be calculated if it is assumed that the ratio of cells to plasma is constant throughout the circulation. The apparent circulating blood volume in milliliters equals the total radioactivity injected (microcuries) divided by the concentration of radioactivity (microcuries/ml.) in the blood samples. Arithmetically this is the equivalent of dividing the

[2] Much of the random fluctuation within a single experiment is apparently due to errors in the radioactive measurements. The size of this error depends upon the intensity of the radiation in the different experiments unless a prohibitive amount of time is taken for counting.

erythrocyte volume by the hematocrit reading of 36°per cent and gives an average value of 53 ± 4 mls./kgm. of body weight.

Blood volumes calculated in this way are only "apparent" because the calculation depends on a constant ratio of cells to plasma throughout the circulation. According to the work of Whipple (8) and collaborators on dogs and of Fåhraeus (9, 10) this is not true, and such blood volumes may be systematically in error whether calculated by means of plasma or erythrocyte volumes.

Hahn and Hevesy (3) report an apparent blood volume of 38 ml/kgm. and 46 ml/kgm. in two rabbits using their method for erythrocytes. An average blood volume in rabbits of 70 ml/kgm. based on the dye method for plasma volume has been reported (11). One of the Hahn and Hevesy values is within the range of the present experiments but the other seems definitely lower. The discrepancy between all of the blood volume values based on erythrocyte and those based on plasma determinations is about the same as that previously found (1) in the dog and ascribed to non-uniform erythrocyte distribution.

TABLE 1

EXPERIMENT	CIRCULATING ERYTHROCYTE VOLUME IN MLS./KGM. OF BODY WEIGHT
1	20
5	17
6	18
7	22
8	18
9	18
10	21
	Average 19 ± 1.4 mls./kgm.

In conclusion, then, the method gives reasonable values for the rabbit as far as the scanty data in the literature go. Previously described methods using radioactive elements obtain cells from donor animals which makes application to humans very difficult. The present method is, with minor changes, now being tried on humans with encouraging results so far.

SUMMARY

The measured loss from circulating rabbit blood of radioactive phosphate, contained in erythrocytes into which it had been introduced in vitro, was found to be fairly low in the period from 5 to 25 minutes after injection. This fact permits the use of such cells as the basis of a relatively simple method for determining circulating erythrocyte volumes in these animals.

REFERENCES

(1) HAHN, P. F., W. M. BALFOUR, J. F. ROSS, W. F. BALE, AND G. H. WHIPPLE.   Science 93: 87, 1941.

(2) HAHN, P. F., J. F. ROSS, W. F. BALE, W. M. BALFOUR AND G. H. WHIPPLE.   J. Exper. Med. 75: 221, 1942.

(3) HAHN, L. AND G. HEVESY.   Acta Physiol. Scand. 1: 3, 1940.

(4) HALPERN, L.   J. Biol. Chem. 114: 747, 1936.

(5) ATEN, A. H. W. AND G. HEVESY.   Nature 142: 871, 1938.

(6) HEVESY, G. AND A. H. W. ATEN.   Kgl. Danske Videnskab. Selskab. Biol. Medd. 14: no. 5, 1939.

(7) EISENMAN, A. J., L. OTT, P. K. SMITH, AND A. W. WINKLER.   J. Biol. Chem. 135: 165, 1940.

(8) SMITH, H. P., H. R. ARNOLD AND G. H. WHIPPLE.   This Journal 56: 336, 1921.

(9) FÅHRAEUS, R.   Physiol. Rev. 9: 241, 1929.

(10) FÅHRAEUS, R. AND T. LINDQUIST.   This Journal 96: 562, 1931.

(11) LEVINE, C. J., W. MANN, H. C. HODGE, I. ARIEL AND O. DUPONT.   Proc. Soc. Exper. Biol. and Med. 47: 318, 1941.

EFFECT OF PURIFIED PITUITARY PREPARATIONS ON URINE NITROGEN IN THE RAT[1]

WALTER MARX, DAVID B. MAGY, MIRIAM E. SIMPSON AND HERBERT M. EVANS

From the Institute of Experimental Biology, University of California, Berkeley

Teel, Gaebler and Lee and their co-workers observed several years ago that pituitary extracts caused nitrogen retention in dogs (1, 2) and rats (3). Since the pituitary preparations available at the time were only partially purified, consisting of combinations of several of the pituitary hormones, it was not possible to attribute unequivocally the observed effects to the action of any one of the different constituents of pituitary extracts. Sufficient advance has recently been made in the separation of the six generally recognized pituitary hormones, so that one can now attempt to determine which components of pituitary extracts influence nitrogen metabolism. The present paper reports experiments in which highly purified pituitary hormone preparations were compared as to their action on urine nitrogen in rats.[2]

EXPERIMENTAL. *Method.* Total urinary nitrogen excretion was measured in normal "plateaued" female rats during a period of 24 hours. For collection of the urine, the animals were kept in cages equipped with screen bottoms, and a pan of corresponding size was put under each cage, containing a sheet of filter paper moistened with a solution of 3 per cent benzoic acid in 50 per cent alcohol as preservative (5). After termination of the collection period, feces were removed from the filters, and pan and filters washed repeatedly with hot water. The combined washings of each sample were made up to 1 liter, and then 1.0 cc. samples taken for micro Kjeldahl determinations of total nitrogen.

It was considered important to keep food consumption identical for both experimental and control rats. Two series of experiments were carried out. In the first, the rats were kept four in a cage, and they were fasted during the experimental period. In the second series the animals were kept in smaller cages, one rat per cage, and each animal of both experimental and control groups received 7 grams' diet[3], an amount which is only a fraction of the average daily normal food consumption. Any animal which did not eat the whole amount of food, was not included in the results. The food was given in glass containers with a special top to prevent loss through scattering.

In one series of experiments, the effect of hormone treatment was to be studied

in the absence of the thyroid gland. For this purpose, female rats were thyroidectomized[4] at 53 to 54 days of age[5], and used about a month later for the experiments. Completeness of the operation was checked by determining the metabolic rate of the thyroidectomized animals; retardation of growth served as additional criterion. The site of operation was also carefully examined at autopsy for gland fragments.

Hormone preparations. The hormone preparations were administered in saline solution, in two intraperitoneal injections, 4–5 hours apart, the first about one hour previous to the onset of the period of urine collection.

The following hormone preparations were used: 1. Alkaline extracts of beef anterior pituitary, containing all known pituitary hormones. 2. "Globulin fractions", obtained by precipitation at 0.5 saturated ammonium sulfate (6). These fractions contained most known pituitary hormones, with exception of the follicle stimulating hormone. 3. Purified pituitary growth hormone preparations having a minimal effective dose of about 30 micrograms (daily, 10 day test in hypophysectomized rats) (7). In these preparations, follicle stimulating, interstitial cell stimulating, adrenocorticotropic and thyrotropic hormones were not demonstrable at 5 mgm. (total dose, 10 day test in hypophysectomized rats), and lactogenic hormone not at 20 mgm. (total dose, systemic crop test in squabs). 4. Purified pituitary thyrotropic hormone preparations[6], having a minimal effective dose of 20 micrograms (total dose, 5 day test in chicks) (8). In these preparations, interstitial cell stimulating hormone was demonstrable at about 0.10 to 0.15 mgm. (total dose, 3 day test in hypophysectomized rats), and small amounts of growth promoting activity at 5 mgm. (total dose, 10 day test in hypophysectomized rats). However, assays of thyrotropic hormone preparations for growth hormone contamination are equivocal, since thyroxin causes similar insignificant body weight increases; when given to hypophysectomized rats at "corresponding" doses[7], and in view of the synergism between growth hormone and thyrotropic hormone reported recently (9). Therefore, the apparent growth hormone contamination of about 1 per cent ought to be considered only as a maximum value. 5. Purified pituitary lactogenic hormone preparations[8], having a potency of about 30 international units per milligram (10). In these preparations no gonadotropic or thyrotropic hormones were demonstrable at 100 mgm. (total dose, 10 day test in hypophysectomized rats), but a small amount of adrenocorticotropic activity. It is problematical whether an insignificant body weight gain resulting occasionally at these high dose levels was due to a contamination with growth hormone. 6. A purified pituitary follicle stimulating hormone preparation[9] which had a minimal effective dose of 20 micrograms (total dose, 3 day test in hypophysectomized rats) and whose only

[4] Thyroidectomies were kindly performed by Dr. W. O. Reinhardt.
[5] Only 4 out of 31 animals were about 8 months old at operation.
[6] Kindly supplied by J. Fraenkel-Conrat.
[7] Dose levels comparable in their ability to raise the oxygen consumption in the hypophysectomized rat. (H. Fraenkel-Conrat and V. V. Herring, unpublished.)
[8] Kindly supplied by Dr. W. R. Lyons.
[9] Kindly supplied by Dr. H. Fraenkel-Conrat.

contamination was interstitial cell stimulating hormone (11).   7. A purified pituitary interstitial cell stimulating hormone preparation[10] which had a minimal effective dose of 10 micrograms (daily dose, 3 day test in hypophysectomized rats), and which was practically free of all other recognized pituitary hormones (12).   8. Commercial preparations of thyroxin, adrenal cortex extract and protamine zinc insulin.[11]

RESULTS.   The changes in urinary nitrogen excretion produced in fasting rats by successive dilutions of the "alkaline extract" and of the "globulin fraction" are shown in table 1.   The data of this series were not treated statistically, since only 3 separate urine samples were obtained per group in each experiment (3 cages per group, 4 rats per cage pooled).   The results summarized in table 1 indicate a drop in nitrogen excretion up to 32 per cent, which is in agreement with observations of previous authors (1, 2, 3).   The response decreased with decreasing dose, but the correlation was not sufficient to provide a quantitative method for assay of the active principle.   Such a high variation has been reported previously (2, 13).

The results are, however, consistent enough to justify use of the method to investigate which components of pituitary extracts are responsible for the observed effects.   For this purpose the nitrogen retaining power of highly purified pituitary hormone preparations was determined in fasted rats at a dose of 5 mgm. This was the lowest dose level at which the globulin "fraction" had caused significant effects in fasted rats.   The results, summarized in table 2, indicate that, of the purified pituitary hormones, growth hormone had the most marked effect. A smaller decrease in urinary nitrogen was produced by thyrotropic hormone, while lactogenic hormone caused a reduction of only 13 per cent, a result whose significance is questionable.   That the observed effects are specific and not given by any protein, is shown by the negative results obtained after injection of 5 mgm. follicle stimulating hormone or 20 mgm. casein.   Thyroxin and adrenal cortex extract had no effects at the dose levels tested.

In the second series of experiments, carried out with paired feeding, the amount of excreted nitrogen was larger and the sensitivity to injected hormones higher than in fasted rats.   Under these conditions, the lowest dose of the globulin fraction which caused significant effects was estimated to be one milligram. The purified pituitary hormones were, therefore, injected at 1 mgm. doses.   As the rats were kept in separate cages, the number of urine samples collected separately was sufficient for calculation of standard errors.   A result was considered significant when $D = 3 \times S_D$ (D = difference in urinary nitrogen excretion between experimental and control rats, S_D = standard error of the difference). Table 3 shows that growth hormone again had the most marked effect on nitrogen retention.   Thyrotropic hormone was next in potency causing a statistically significant decrease in urine nitrogen.   Lactogenic hormone produced a 15 per

cent drop which was, however, not statistically significant. Interstitial cell stimulating hormone, thyroxin and insulin had no effect at the levels tested.

Experiments carried out in a similar manner with thyroidectomized rats are summarized in table 4 which indicates that both thyrotropic and growth hormones reduced urine nitrogen in the absence as well as in the presence of the thyroid gland.

TABLE 1

Effect of alkaline pituitary extract and "globulin fraction" on urine nitrogen in the fasted normal "plateaued" female rat

Relationship of dose to response

PITUITARY PREPARATION	TOTAL DOSE	NUMBER OF RATS		PER CENT CHANGE IN URINE NITROGEN
		Injected	Controls	
	mgm.			
Alkaline extract	20.0	12	14	−32
	10.0	24	26	−26
	5.0	12	12	−24
	2.5	12	12	−15
"Globulin fraction"	20.0	11	16	−22
	10.0	12	12	−21
	5.0	60	56	−19
	2.5	12	12	−10

TABLE 2

Effect of purified pituitary hormone preparations, thyroxin and adrenal cortex extract on urine nitrogen in the normal "plateaued" female rat

Injected and control rats fasted

HORMONE PREPARATION	TOTAL DOSE	NUMBER OF RATS		PER CENT CHANGE IN URINE NITROGEN
		Injected	Controls	
	mgm.			
Growth hormone.....................	5.0	32	36	−24
Thyrotropic hormone.................	5.0	24	24	−19
Lactogenic hormone..................	5.0	12	12	−13
Follicle stimulating hormone..........	5.0	12	12	−3
Thyroxin............................	0.2	11	12	−5
Adrenal cortex extract...............	1.0 cc.*	24	20	−7
Casein..............................	20.0	12	16	−5

* 1.0 cc. adrenal cortex extract contains "not less than 2.5 rat units."

DISCUSSION. The results summarized in tables 2 and 3 show that purified growth hormone preparations had the greatest effect on nitrogen retention of any of the known pituitary hormones. · This confirms the concept of earlier workers (1, 2, 3, 13) that growth hormone is the pituitary factor responsible for a positive nitrogen balance.

It can be seen, however, from tables 2 and 3 that growth hormone was not the

only component of unfractionated pituitary extracts which reduced urine nitrogen. Purified thyrotropic hormone also caused significant nitrogen retention, though the effect was smaller than that of growth hormone. Since purified thyrotropic preparations cause only insignificant body weight increases in hypophysectomized rats, this finding was rather unexpected, and it was considered important to investigate, whether the action of the thyrotropic hormone preparation on nitrogen retention was mediated through the thyroid gland. For this

TABLE 3

Effect of purified pituitary hormone preparations, thyroxin and protamine zinc insulin on urine nitrogen in the normal "plateaued" female rat

Injected and control rats on identical food intake

HORMONE PREPARATION	TOTAL DOSE	NUMBER OF RATS		PER CENT CHANGE IN URINE NITROGEN AND STANDARD ERROR
		Injected	Controls	
	mgm.			
Growth hormone.....................	1.0	26	23	−24 ±5
Thyrotropic hormone.................	1.0	5S	57	−17 ±5
Lactogenic hormone..................	1.0	3S	39	−15 ±6
Interstitial cell stimulating hormone..	1:0	S	7	−5 ±3
Thyroxin...........................	0.1	12	12	−4 ±4
Thyroxin...........................	0.03	12	12	+4 ±6
Protamine zinc insulin...............	2.0*	10	15	−7 ±6

* Units.

TABLE 4

Effect of purified pituitary thyrotropic and growth hormones on urine nitrogen in the thyroidectomized rat

Injected and control rats on identical food intake

HORMONES INJECTED	TOTAL DOSE MGM. PER 100 GRAMS BODY WEIGHT*	NUMBER OF RATS		PER CENT CHANGE IN URINE NITROGEN
		Injected	Controls	
Thyrotropic hormone..............	0.4	16	15	−15
Growth hormone..................	0.4	7	8	−22

* Since rats of two different weight groups were used, the hormones were administered in proportion to their body weights.

purpose two series of experiments were performed. The action of thyrotropic hormone was studied in thyroidectomized rats, and the effect of thyrotropic hormone in normal animals was compared with that of thyroxin. The results obtained with thyroidectomized rats indicate that purified thyrotropic hormone decreased urine nitrogen even in the absence of the thyroid gland, as far as can be judged from the limited number of animals (table 4). On the other hand, thyroxin, given to normal rats at "corresponding" levels had no effect on nitrogen retention (tables 2 and 3). (This finding does not represent a contradiction to

the well established fact that high dose levels of thyroid hormone cause nitrogen loss (14), since in the present work only relatively low dose levels were administered.) These results support the concept that the action of thyrotropic hormone on nitrogen retention is not mediated through the thyroid gland.

The action of thyrotropic hormone on urinary nitrogen excretion can be correlated with similar effects on blood nonprotein nitrogen. Blood urea and α-amino acid levels in particular are lowered by this hormone, as found recently by J. Fraenkel-Conrat et al. (4). Blood α-amino acids are decreased in the absence as well as in the presence of the thyroid gland.

It is problematical whether the observed effects of thyrotropic hormone preparations on nitrogen metabolism which are not mediated by the thyroid gland are due to an intrinsic property of the thyrotropic hormone itself, or to another hormone contaminating the thyrotropic preparations. These preparations are known to contain some interstitial cell stimulating hormone and probably less than 1 per cent growth hormone. The former can be eliminated, since purified interstitial cell stimulating hormone had no effect on urine nitrogen (table 3). The amount of growth hormone present in thyrotropic hormone preparations is probably too small to account for the observed effects. The question whether the action is due to the thyrotropic hormone itself, or to an unknown contamination is, however, still open, and can only be settled when the thyrotropic hormone is isolated in pure form.

The effect of purified pituitary lactogenic hormone on nitrogen retention was even smaller than that obtained with thyrotropic hormone, and the average standard error of this group of experiments was so large that the results cannot be regarded statistically significant according to the criterion defined. However, since the lowering of urine nitrogen was observed repeatedly and, since the effect was consistently larger than that elicited by any of the remaining hormones examined (the pituitary follicle stimulating and interstitial cell stimulating hormones, thyroxin, insulin and adrenal cortex extract) it seems inadvisable completely to disregard this action of lactogenic hormone on nitrogen balance. Perhaps study of urinary nitrogen excretion in hypophysectomized rats may bring greater clarity on this point.

SUMMARY

The action of anterior pituitary hormones on urinary nitrogen excretion was studied in the normal "plateaued" female rat.

Unfractionated pituitary extracts caused a marked decrease in urine nitrogen.

Purified pituitary growth hormone preparations induced a similar drop in urinary nitrogen excretion.

Purified pituitary thyrotropic hormone preparations caused a significant but smaller reduction in urine nitrogen. This action is probably not mediated by the thyroid gland.

Purified pituitary lactogenic hormone preparations had an even smaller effect on nitrogen retention, statistically not significant, although consistently larger than that obtained with the remaining hormones examined.

Purified pituitary follicle stimulating and interstitial cell stimulating hormone preparations, thyroxin, adrenal cortex extract and protamine zinc insulin, at the dose levels tested, were without effect on urinary nitrogen excretion.

REFERENCES

(1) TEEL, H. M. AND H. CUSHING. Endocrinology **14**: 157, 1930.

(2) GAEBLER, O. H. J. Exper. Med. **57**: 349, 1933.

(3) LEE, M. O. AND N. K. SCHAFFER. J. Nutrition **7**: 337, 1934.

(4) FRAENKEL-CONRAT, J., H. FRAENKEL-CONRAT AND H. M. EVANS. This Journal, **137**: 200, 1942.

(5) ASHWORTH, U. S. AND S. BRODY. Univ. Missouri, Agric. Exper. Sta., Res. Bull. **189**: 1933.

(6) EVANS, H. M., N. UYEI, Q. R. BARTZ AND M. E. SIMPSON. Endocrinology **22**: 483, 1938.

(7) FRAENKEL-CONRAT, H. L., D. L. MEAMBER, M. E. SIMPSON AND H. M. EVANS. Endocrinology **27**: 605, 1940. (A modification of the procedure will be published in the near future.)

(8) FRAENKEL-CONRAT, J., H. FRAENKEL-CONRAT, M. E. SIMPSON AND H. M. EVANS. J. Biol. Chem. **135**: 199, 1940.

(9) MARX, W., M. E. SIMPSON AND H. M. EVANS. Proc. Soc. Exper. Biol. and Med. **49**: 594, 1942.

(10) LYONS, W. R. Proc. Soc. Exper. Biol. and Med. **35**: 645, 1937.

(11) FRAENKEL-CONRAT, H., M. E. SIMPSON AND H. M. EVANS. Anales de la Facultad de Medicina, Montevideo **25**: nos. 5, 6, 7 and 8, 1940.

(12) LI, C. H., M. E. SIMPSON AND H. M. EVANS. Endocrinology **27**: 803, 1940.

(13) LEE, M. O. Assoc. Research Nerv. Mental Dis. **17**: 193, 1938.

(14) ROOS, E. Ztschr. Physiol. Chem. **21**: 19, 1896.

EXPERIMENTAL HUMAN VITAMIN A DEFICIENCY AND THE ABILITY TO PERFORM MUSCULAR EXERCISE

GEORGE WALD, LUCIEN BROUHA AND ROBERT E. JOHNSON[1]

From the Biological Laboratories and the Fatigue Laboratory of Harvard University

In the course of studies on experimental human vitamin A deficiency one of us (G. W.) held five young men on a diet very poor in vitamin A for a period of about six months. Measurements were made at regular intervals of the subjects' cone and rod dark adaptation, the vitamin A and total carotenoid content of the plasma, and the carotene and xanthophyll content of the feces. The results of these experiments are abstracted in the present paper and will be presented in detail elsewhere.

It seemed desirable to examine these subjects also as part of a program under way at the Fatigue Laboratory to determine the effects of various types of diet on human physical fitness and fatigue. Two series of exercise measurements were therefore performed upon the subjects, one after several months on the deficient diet, the second some six weeks after the return to a normal diet. The present paper is concerned primarily with the results of these latter measurements.

METHODS. The subjects were medically normal college students. During the entire experiment they maintained their ordinary classroom routines, living for the most part a sedentary existence. For 30 days before going on the deficient diet all of them supplemented their normal diets with about 75,000 I.U. of vitamin A daily. They therefore began the deficient diet with what were probably very high reserves of vitamin A.

Four of the subjects started upon the vitamin A-low diet abruptly on November 4 to 6, 1940; the fifth (Al) on December 2; and all of them remained on this diet for almost exactly six months. In general they were advised to eliminate from the diet all colored vegetable material, all dairy products except fat-free milk, and liver and kidney. They were guided in immediate detail by the food charts of Eddy and Dalldorf (1), copies of which were distributed to them, and used as principal further reference the tables of Daniel and Munsell (2). An attempt was made to keep the total vitamin A intake within about 100 I.U. per day. This may be compared with the estimated minimal requirement of about 1500 I.U. daily, and the daily allowance of 5000 I.U. per day recently recommended by the Committee on Food and Nutrition of the National Research Council (3).

This vitamin A-low diet was supplemented daily with the following factors: brewer's yeast containing at least 140 I.U. of vitamin B_1 and 70 Sherman Units

of B_2; 50 mgm. of ascorbic acid; about 1300 U.S.P. units of vitamin D (irradiated ergosterol); 2 grams of dicalcium phosphate; and a preparation containing 32 mgm. of iron and 0.64 mgm. of copper as gluconates.[2]

Except for small and temporary decreases in weight which in no case exceeded five pounds, all the subjects maintained their initial weights. Even these transient decreases, which appeared due to lowered caloric intake in the period just after the diet had been altered, were repaired within a few weeks on the deficient diet.

Two or three times each week throughout the entire period of the experiment the complete dark adaptation curve was measured by methods previously described (4). About once a week samples of blood were drawn and the carotenoid and vitamin A content of the plasma were determined. At least once each week a sample of feces was analyzed for carotene and xanthophyll. Since it has been shown that under the best conditions for absorption about 60 per cent of ingested carotene is excreted in the feces, these measurements yielded an objective check on the carotene content of the diet (5).

After the subjects had been on the deficient diet for 3 to $4\frac{1}{2}$ months their ability to perform moderate and heavy muscular exercise was determined with the standard Fatigue Laboratory treadmill test: 15 minutes' walk at 3.5 M.P.H. on an 8.6 per cent grade; 10 minutes' rest; run to exhaustion or up to 5 minutes at 7 M.P.H. on an 8.6 per cent grade; and recovery followed for 20 minutes after the run.

Measurements were made of the sitting heart rate and blood pressure before the walk; continuous heart rate during and after the walk and the run; ventilation during the walk and the run; oxygen consumption during the walk and the run; and blood lactate during the walk and after the run.

At the close of the period on the vitamin A-low diet the subjects returned to a normal diet supplemented with vitamin A. After at least six weeks of this they were tested on the treadmill as before.

OBSERVATIONS. Within one week after starting the deficient diet the feces carotene of all subjects had fallen to about one per cent of its previous values. Shortly afterward the plasma carotenoids were found to have decreased from 1 to 2 μgm. per cc.—the normal level—to at most about 0.5 μgm. per cc. and in some instances traces which could not be measured accurately. These factors follow the immediate diet closely.

The plasma vitamin A however maintained its initial maximal levels (1.57 to 2.97 I.U. per cc.) in all subjects throughout the entire period on the deficient diet. The threshold of the completely dark adapted rods—the first to rise in vitamin A deficiency (4)—also remained constant and minimal in three subjects throughout the course of the experiment. In two subjects it rose very slowly

on the deficient diet: in McA about 1.1 log unit and in Ms about 0.7 log unit in all. Three subjects therefore gave no objective evidence of vitamin A deficiency, the other two only moderate increases in visual threshold following six months of vitamin A deprivation.

At the close of the deficiency period the subject McA was given 25,000 I.U. of vitamin A as halibut liver oil orally. Four hours later his final rod threshold had fallen about 0.3 log unit. He remained on the deficiency diet, supplemented with 25,000 I.U. of vitamin A daily. After 15 days his threshold had descended a total of 0.75 log unit, and was still about 0.35 log unit above normal. The vitamin A supplement was increased to 75,000 I.U. daily; after another 11 days the threshold had reached normal. At the end of the deficiency period Ms was given 50,000 I.U. of vitamin A orally in the form of carotene in cottonseed oil. After 80 minutes, and on testing again after five days, his threshold had not changed appreciably. He returned to his normal diet supplemented with 60,000 I.U. of carotene daily. After 28 days his threshold still had not changed significantly.

These results are comparable with the slow cures of experimental night-blindness observed under almost identical conditions by Hecht and Mandelbaum (6) and by McDonald and Adler (7). They differ strikingly from the very rapid reversals of night-blindness frequently obtained in similar experiments and apparently prevalent under clinical and field conditions (8). They indicate some deep-seated lesion the development of which may be peculiarly favored by the conditions of the present type of experiment—perhaps the sudden and acute onset and long continuance of the deficient diet, and the sharp restriction of the deficiency to vitamin A alone.

The results of the treadmill experiments follow:

A. *Reaction to moderate muscular work (walking).* In measurements made while the subjects were on the vitamin A-low diet no significant differences from normal appeared either during or following mild muscular work. Specifically there was practically no difference between these and the post-deficiency measurements with respect to heart rate during and after the walk; blood pressure after the walk; and ventilation, oxygen consumption, respiratory quotient, and blood lactate during the walk.

B. *Reaction to exhausting exercise (running).* The deficient diet appeared to have no significant effect on the ability to perform exhausting exercise of short duration. Specifically there were no definitely significant differences between the deficiency and post-deficiency measurements of duration of effort; heart rate during and after the run; blood pressure after the run; ventilation, oxygen consumption and respiratory quotient during the run; and blood lactate immediately after the run.

C. *Quantitative indices of fitness for moderate and for exhausting exercise.* Indices of fitness based upon significant physiological variables have recently been proposed by Johnson and Brouha (9) and by Johnson, Brouha and Darling (10). The "work index" is defined as the duration of effort in seconds minus the maxi-

mal heart rate during the effort plus the blood lactate. The "recovery index" is defined as:

$$\frac{\text{Duration of effort in seconds} \times 100}{2 \times \text{sum of pulses from } 1\text{--}1\frac{1}{2}, \ 2\text{--}2\frac{1}{2}, \text{ and } 4\text{--}4\frac{1}{2} \text{ minutes during recovery}}$$

Both indices rise with improvement in performance. It should be emphasized however that only in exhausting work do they allow accurate ranking of subjects in comparison with one another. Under such conditions "work indices" above
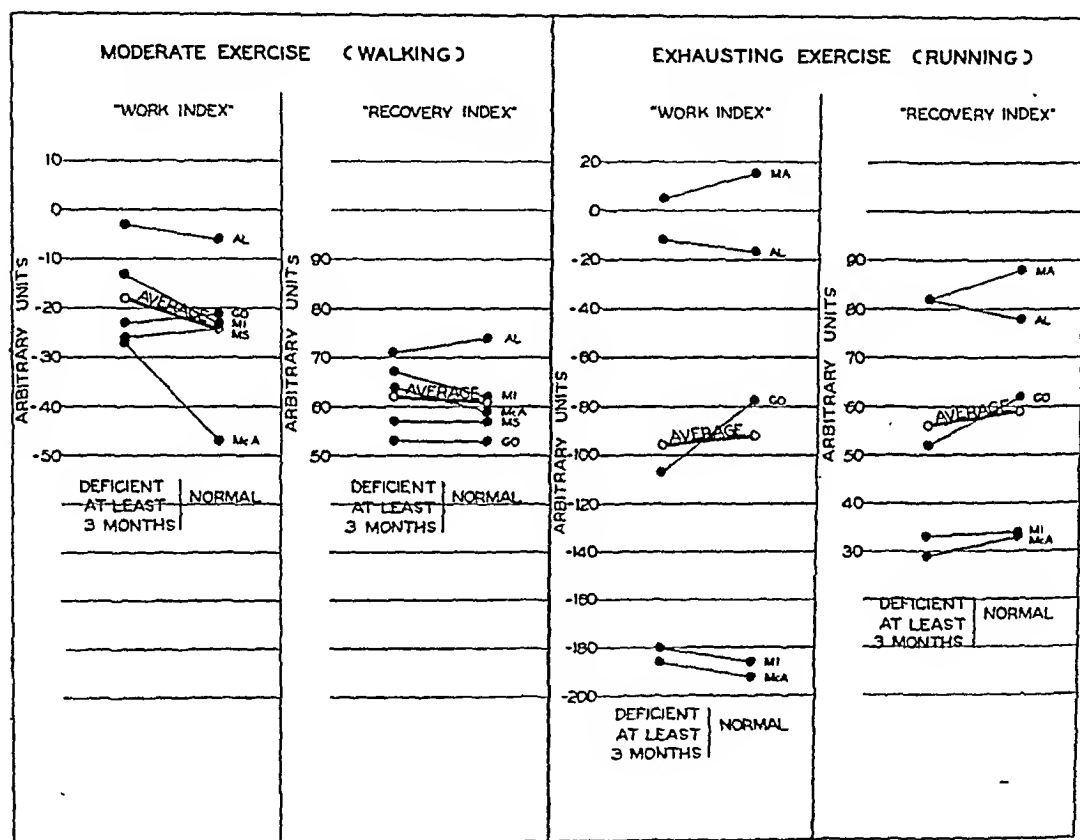
Fig. 1. Work and recovery indices in mild and exhausting exercise for five subjects 3–4½ months on a vitamin A-deficient diet, and about six weeks after the return to a normal diet supplemented with vitamin A.

−50 are good; from −50 to −150 are average; and below −150 are poor. "Recovery indices" above 75 are good; 40 to 75 are average; below 40 are poor. In moderate exercise these indices are valuable only for comparing the same subject with himself from time to time.

Figure 1 shows these indices for the present subjects in the deficiency and post-deficiency periods. The walk indices show *no significant changes* except in one subject (McA) for whom they were lower on the normal diet. The indices for running show *no significant change* in any subject. It is noteworthy that of the

two subjects who yielded some positive evidence of vitamin A deficiency—rise in visual threshold—Ms yielded the highest and McA the lowest indices in both periods. The slight changes in the scores of these subjects in the two test periods lie within the ordinary week-to-week variation found in persons on a normal diet. Subject Go resumed his usual spring athletic activities shortly after the first test had been performed, and his slightly improved score in the second test was probably the result of training.

D. *Subjective impressions.* During the period of deficiency only one subject (McA) complained of abnormal fatigue and lassitude. This was the subject who also exhibited the greatest rise in visual threshold. After returning to a normal diet he reported feeling definitely better, yet he performed no better than previously on the treadmill.

The remaining subjects reported no special impressions during the deficiency period. After resuming the normal diet they all declared that they felt better and that the running test on the treadmill was easier. Yet only two of them showed any actual improvement, very slight in both cases.

DISCUSSION. Following a period of high vitamin A nutrition which permits the accumulation of large internal reserves, sedentary human subjects can tolerate almost total deprivation of vitamin A for periods as long as six months without developing objective or subjective evidences of deficiency. The maintenance of plasma vitamin A at its maximal levels in all subjects throughout this interval implies that at its close the internal reserves were still sufficient to keep the tissues optimally supplied. Yet it is significant that two of the subjects who displayed no measurable decrease in plasma vitamin A still exhibited moderate increases in the visual threshold. This is just the reverse phenomenon of that recently reported by Bodansky, Lewis and Haig (11), in which the final rod threshold was normal in cases exhibiting low plasma vitamin A. Apparently either situation may occur, depending upon circumstances not yet wholly appreciated or controlled.

Neither the subjects who exhibited rise in visual threshold nor the others in the present experiments displayed any significant impairment in the ability to perform exhausting exercise after 3 to $4\frac{1}{2}$ months on the deficient diet. It is of course possible that men doing hard physical labor or exposed to rigorous environmental conditions such as extreme heat or cold might have responded differently. In any case the results of these experiments are in marked contrast with those obtained with sedentary subjects deprived of the vitamin B complex, who showed within three weeks symptoms of abnormal fatigability and measurable physical deterioration (12).

SUMMARY

1. Following 30 days of high vitamin A nutrition, five young men were maintained on a diet extremely low in vitamin A for a period of about six months. The A-deficient diet was supplemented with brewer's yeast, ascorbic acid, vitamin D, calcium phosphate, iron and copper. At regular intervals the cone and rod dark adaptation were measured, and the concentrations of vitamin A and

total carotenoid in the plasma, and of carotene and xanthophyll in the feces were determined.

2. Within a week on the A-deficient diet the fecal carotenoids had fallen to about 1 per cent of their previous values. Shortly afterward the plasma carotenoid also was found at very low levels. The plasma vitamin A levels, however, maintained their initial maximal values throughout the entire deficiency period. The visual thresholds of three of the subjects also remained constant and minimal; in two subjects they rose slowly to final levels 0.7–1.1 log unit above normal.

3. After 3 to $4\frac{1}{2}$ months on the deficient diet, and again about six weeks after the return to a normal, A-supplemented diet, the physical fitness of the subjects for moderate and exhausting exercise was determined. No significant differences in performance were found.

4. It is concluded that relatively sedentary subjects initially well supplied with vitamin A may undergo as much as six months of vitamin A deprivation without developing objective or subjective evidences of vitamin A deficiency.

REFERENCES

(1) EDDY, W. H. AND G. DALLDORF. The avitaminoses. Williams & Wilkins, Baltimore, 1938, pp. 316–321.
(2) DANIEL, E. P. AND H. E. MUNSELL. U. S. Dept. of Agriculture Misc. Publ. no. 275, 1937.
(3) Committee on Food and Nutrition of the National Research Council, J. A. M. A. **116**: 2601, 1941.
(4) WALD, G., H. JEGHERS AND J. ARMINIO. This Journal **123**: 732, 1938.
(5) WALD, G., W. R. CARROLL AND D. SCIARRA. Science **94**: 95, 1941.
(6) HECHT, S. AND J. MANDELBAUM. This Journal **130**: 651, 1940.
(7) McDONALD, R. AND F. H. ADLER. Arch. Ophthal. **24**: 447, 1940.
(8) STEVEN, D. AND G. WALD. J. Nutrition **21**: 461, 1941.
(9) JOHNSON, R. E. AND L. BROUHA. Rev. Canad. Biol. **1**: 171, 1942.
(10) JOHNSON, R. E., L. BROUHA AND R. C. DARLING. Rev. Canad. Biol., **1**: 491, 1942.
(11) BODANSKY, O., J. M. LEWIS AND C. HAIG. Science **94**: 370, 1941.
(12) EGAÑA, E., R. E. JOHNSON, R. BLOOMFIELD, L. BROUHA, A. P. MEIKLEJOHN, J. WHITTENBERGER, R. C. DARLING, C. HEATH, A. GRAYBIEL, AND F. CONSOLAZIO, This Journal, in press.

THE RELATION OF DIETHYL-STILBESTROL TO CARBOHYDRATE METABOLISM IN ADRENALECTOMIZED AND HYPOPHYSECTOMIZED RATS[1]

RALPH G. JANES AND WARREN O. NELSON

From the Department of Anatomy, Wayne University College of Medicine, Detroit, Mich.

In previous studies from this laboratory it has been shown that the level of liver glycogen increases in normal rats which have been treated with diethyl-stilbestrol (1) and that this increase is more pronounced in castrates than in normal rats (2). These observations were confirmed by Griffiths, Marks and Young (3), and Ingle (4) has reported that the administration of stilbestrol to normal non-glycosuric rats frequently produced a mild glycosuria.

The question arises as to the manner in which stilbestrol produces these effects on carbohydrate metabolism. Since it seems assured that it does not act through the gonads (2) the possibility exists that it may act directly upon carbohydrate metabolism or indirectly through some organ or organs. In view of the fact that fasting adrenalectomized or hypophysectomized animals show low carbohydrate levels (8, 12) it is possible that stilbestrol acts through the medium of one of these glands. This idea receives support in the fact that stilbestrol mimics the effects of cortical hormone on carbohydrate levels. In an examination of this possibility, stilbestrol was administered to a series of animals in which the adrenals or pituitaries had been removed. This study has been reported in preliminary form (Janes, 5).

METHODS. Observations on 32 adrenalectomized rats of the Sprague-Dawley strain are reported. When adrenalectomy was performed the periadrenal fat was excised widely to insure the removal of adjacent accessory adrenal tissue. Our experience with this strain has shown that accessory tissue occurs occasionally, but with much less frequency than in most other strains. Following the operation, the rats were given Rubin-Krick solution and, in addition, some groups of animals were maintained on 3 mgm. desoxycorticosterone acetate (DCA)[2] daily or on adrenal cortical extract[3], ½ cc. twice daily. These doses, particularly for DCA, are more than enough to maintain otherwise untreated adrenalectomized animals in good health, but relatively huge amounts of cortical hormone are required in adrenalectomized animals which are under stilbestrol treatment. Subcutaneous injections of stilbestrol[4] (100 micrograms daily) were initiated about 3 days following operation and were continued for 16 to 18 days.

Hypophysectomized rats were given 5 per cent glucose as drinking water and

fed a stock diet of pellets supplemented by greens. Treatment with stilbestrol (50 micrograms daily) was started three days after the operation and was continued for 14 days.

At the end of the experiment, adrenalectomized animals were fasted for 24 hours and hypophysectomized animals for 12 hours. The last injections were given on the day preceding the fast. Immediately before the animals were anesthetized with sodium amytal, blood samples were taken from the tail vein for determinations of blood sugar by the Shaffer-Hartmann-Somogyi micro-method. Following anesthetization, the gastrocnemii were dissected free and, together with samples of liver, were frozen quickly in a mixture of carbon dioxide and ether and prepared for the determination of glycogen levels by the method of Good, Kramer and Somogyi (11). The gonads, adrenals and pituitaries, when present, were removed, weighed and fixed for microscopic examination. Urine was collected in 5 per cent sulphuric acid during the fasting periods and was used for the determinations of non-protein nitrogen by the micro-Kjeldahl method.

RESULTS. A. *Effects of stilbestrol on adrenalectomized rats.* Three series of experiments were set up to test the effect of stilbestrol on adrenalectomized rats. The first series included untreated controls and animals treated with stilbestrol, but not maintained with cortical hormone. In the second series, the rats received the same treatment with the addition of Eschatin. The animals of the third group received stilbestrol and were maintained with desoxycorticosterone acetate. Controls, which did not receive stilbestrol, were studied in each series (table 1).

The mortality rate of the rats in series 1 was very high. Of 15 untreated adrenalectomized control animals only 6 survived for 18 days. Only 4 of 21 adrenalectomized animals which were treated with stilbestrol survived for 18 days. When $\frac{1}{2}$ cc. Eschatin twice daily (series 2) or 3 mgm. desoxycorticosterone acetate (series 3) was administered it was possible to counteract the effects of stilbestrol and to maintain the animals throughout the experimental period.

Blood sugar levels were low in untreated adrenalectomized rats but were somewhat improved in animals which received injections of cortical hormone. In animals which were treated with both stilbestrol and cortical hormone the blood sugar levels were low.

All adrenalectomized animals, regardless of treatment, showed low values for liver glycogen at the end of a 24 hour fast. Rats which did not receive cortical therapy showed reduced muscle glycogen values (table 1, series 1), while rats which received this therapy, especially those treated with DCA, showed normal or slightly elevated muscle glycogen values. The reason for this effect is not clear.

Although extreme care was used in removing the adrenals and periadrenal fat, in a few rats some hyperplasia of accessory adrenal cortical tissue was observed. This accessory tissue was not sufficient, however, to maintain life or to preserve normal fasting carbohydrate levels.

Urinary nitrogen values showed considerable variation in each series of adrenalectomized animals (table 1). It should be noted that treatment with stilbestrol

produced no constant or significant changes in urinary nitrogen. This is in contrast to the results observed in intact or castrated animals which have been injected with stilbestrol for comparable periods (2).

The weight of the pituitaries was not affected by adrenalectomy in the control animals of series 1. However, the pituitaries of animals treated with cortical extract or DCA showed an increase in size and those of all animals which received stilbestrol were even larger.

TABLE 1

Averages of adrenalectomized rats treated with 100 micrograms diethyl-stilbestrol daily for 16–18 days

NUMBER OF ANIMALS	TREATMENT		WEIGHT AT END OF 24-HR. FAST	BLOOD SUGAR	GLYCOGEN		URINARY N LAST 24 HR.	WEIGHT OF PITUITARY	WEIGHT (2) OF TESTES	WEIGHT (2) OF OVARIES
	Cortical hormone	Stilbestrol			Liver	Muscle				
			grams	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm./ grams†*	*grams/ 100 grams body weight*	*grams/ 100 grams body weight*	*grams/ 100 grams body weight*
Series 1 {6	None	None	217	53±10*	53	276	0.695	0.0046	0.976 (4)**	0.0235 (2)**
{4	None	Stil.	182	44±14	54	242	0.889	0.0093	0.324 (2)	0.0367 (2)
Series 2 {4	Eschatin ½ cc. 2X daily	None	193	67±14	49	447	0.829	0.0067		0.0238 (4)
{3	Eschatin ½ cc. 2X daily	Stil.	150	47±8	37	417	0.856	0.0094		0.0420 (3)
Series 3 {7	DCA 3 mgm. daily	None	216	58±9	42	452	0.719	0.0060	1.115 (3)	0.0169 (4)
{8	DCA 3 mgm. daily	Stil.	193	42±13	22	532	0.679	0.0088	0.792 (2)	0.0390 (6)

* Standard deviation.
** Numbers in parentheses are number of animals.
† Mgm./grams body weight.

The testes of the adrenalectomized rats were normal in size, but those in animals treated with stilbestrol showed considerable damage and loss in weight. After adrenalectomy the ovaries were reduced in size and showed small follicles, but following treatment with stilbestrol the ovaries were increased in size and exhibited large corpora lutea. The mammary glands of the latter animals showed marked stimulation. In these regards the response to stilbestrol is similar to that seen in normal and thyroidectomized animals (6, 7).

B. *Effect of stilbestrol on hypophysectomized rats.* Stilbestrol produced toxic manifestations when administered to hypophysectomized rats. Accordingly, the dosage was reduced to 50 micrograms daily, the animals were treated for only 14 days, and the fasting period was limited to 12 hours at the end of the experi-

ment. Despite these measures only 12 of 24 operated animals survived the experimental period. Controls for this series included groups of untreated normal and stilbestrol-injected normal animals. These were subjected to the same periods of treatment and fasting as the hypophysectomized rats. In normal animals (series 1, table 2) blood sugar levels were higher in the normal rats than in the rats under treatment with stilbestrol. However, the values for liver glycogen of the animals which had been injected with stilbestrol were higher than in the normal rats. Muscle glycogen was unaltered. Urinary nitrogen values were high for both the control and experimental animals of series 1. The fact that these determinations were made from the total urine that was excreted during the 12-hour fast and calculated on the basis of a 24-hour period may account

TABLE 2
Averages of normal and hypophysectomized rats treated with 50 gamma diethyl-stilbestrol daily for 14 days

NUMBER OF ANIMALS		TREATMENT	WEIGHT AT END OF 12-HR. FAST	BLOOD SUGAR	GLYCOGEN		URINARY N FOR 24 HR.	WEIGHT OF PITUITARY	WEIGHT OF ADRENALS (2)	WEIGHT OF TESTES (2)	WEIGHT OF OVARIES (2)
					Liver	Muscle					
			grams	mgm. per cent	mgm. per cent	mgm. per cent	mgm./ grams body weight	grams†	grams†	grams†	grams†
Series 1	5	Controls	226	98±8*	55	435	1.394	0.0044	0.0146	1.021 (5)**	
	4	Stilbestrol	187	75±12	171	486	1.236	0.0059	0.0219	0.820 (4)	
Series 2	8	Hypophysectomy	174	38±14	22	234	0.835		0.0109	0.406 (4)	0.0217 (4)
	12	Hypophysectomy Stilbestrol	190	54±15	28	290	0.670		0.0089	0.342 (9)	0.0185 (3)

* Standard deviation.
** Numbers in parentheses are number of animals.
† Grams/100 grams body weight.

for the high values. The pituitaries and adrenals were increased in size after treatment with stilbestrol and testes were decreased in size. Mammary glands were stimulated.

Hypophysectomized animals in series 2 were given the same treatment as the normal animals in series 1. Following hypophysectomy the carbohydrate levels were decreased and were not altered significantly by treatment with stilbestrol. Urinary nitrogen was decreased in both treated and untreated hypophysectomized animals.

The adrenals underwent atrophy following hypophysectomy and were not enlarged with stilbestrol treatment. Testes and ovaries were smaller in hypophysectomized rats, a condition which was aggravated only slightly by treatment with stilbestrol. Mammary glands were atrophic.

DISCUSSION. Stilbestrol produced definite toxic manifestations when administered to adrenalectomized or hypophysectomized rats. These effects were evidenced by the inability of the rats to withstand treatment for many days, by their intolerance to long fasts, and in a greater loss of weight than is usually seen in animals treated with stilbestrol. Since continued treatment with stilbestrol in doses of 50 to 100 micrograms daily produces mild anorexia and loss of weight in normal animals, an aggravation of these effects in adrenalectomized and hypophysectomized animals is not surprising.

However, adrenalectomized rats withstood the injections of stilbestrol and were maintained in good physical condition when they were given desoxycorticosterone acetate or Eschatin. It should be noted that about six times as much DCA is required to maintain animals which are also receiving stilbestrol as is required in otherwise untreated adrenalectomized animals. The dosage of Eschatin was not large enough and the treatment was not intense enough to produce the elevation in carbohydrate levels which has been described by Long, Katzin and Fry (9).

The blood sugar and liver glycogen levels were low and variable in all adrenalectomized rats. The administration of Eschatin, and to a lesser extent of DCA, raised the blood sugar levels slightly in otherwise untreated adrenalectomized animals, but had no apparent influence on liver glycogen. In adrenalectomized animals which were maintained on Eschatin or DCA the administration of stilbestrol lowered slightly the values for both blood sugar and liver glycogen. This may be due to the anoretic condition of all animals which are treated for appreciable lengths of time with stilbestrol. Under ordinary circumstances the decreased consumption of food is not reflected in the carbohydrate levels since compensatory mechanisms, which appear to be influenced by adrenal cortical hormones, not only overcome the deficit, but actually increase the levels of carbohydrates (1, 2). However, in the absence of the adrenals these mechanisms are not operative and the primary effect of stilbestrol is uncomplicated by compensatory processes.

Blood sugar and liver glycogen levels were extremely low in fasted hypophysectomized rats and were not altered significantly in animals which were treated with stilbestrol. Muscle glycogen values were decreased in animals treated with stilbestrol and are comparable to the levels observed in adrenalectomized animals which did not receive treatment with DCA or Eschatin. It is apparent that stilbestrol is unable to exert an effect upon carbohydrate metabolism in the absence of the hypophysis.

On the basis of the experiments reported here it is possible to formulate a theory to explain the mechanism whereby stilbestrol raises the levels of carbohydrates in the rat. Since the substance is ineffective in adrenalectomized or hypophysectomized animals it is apparent that each of these glands must be involved in the mechanism. It seems probable that stilbestrol stimulates the hypophysis to release some factor, perhaps the adrenotrophic hormone, which in turn stimulates the adrenal cortex to produce considerable quantities of some cortical hormone or hormones, presumably of the type characterized by having

an atom of oxygen attached to carbon 11. Such cortical hormone or hormones are effective in promoting glyconeogenesis and the deposition of glycogen in the livers of fasting animals. This latter action of cortical hormones has been demonstrated by Britton and Silvette (8) and by Long, Katzin and Fry (9). Further support for the theory presented here is found in the fact that stilbestrol induces a marked enlargement of the adrenals in normal and castrated rats, but is without effect in hypophysectomized animals. The argument may be advanced that the adrenals enlarge in response to the introduction of a toxic substance, but the fact remains that this increase does not occur in hypophysectomized animals. Furthermore, adrenal cortical extracts will maintain carbohydrate levels in hypophysectomized rats (9).

The theory which is offered to account for the corticomimetic action of stilbestrol was suggested in an earlier report (Janes, 5). It is noteworthy that, on the basis of acute experiments, data which confirm and extend this idea were presented by Fry, Miller and Long at the annual meeting of the Association of Internal Secretions in Atlantic City, June 9.

These experiments offer further evidence that adrenal cortical compounds of the desoxycorticosterone type, i.e., those lacking an oxygen atom on carbon 11, do not affect carbohydrate levels (10).

SUMMARY

The effect of diethyl-stilbestrol on carbohydrate metabolism has been studied in adrenalectomized and hypophysectomized rats. The following observations were made and on this basis certain conclusions have been drawn:

1. Fasted adrenalectomized rats show low carbohydrate levels and these levels were not improved following the administration of stilbestrol.

2. When adrenalectomized rats were maintained with desoxycorticosterone acetate or small amounts of Eschatin, the blood sugar values were improved only slightly and liver glycogen levels were unchanged. Muscle glycogen values were normal or slightly elevated. The administration of stilbestrol to adrenalectomized rats maintained with cortical hormone failed to effect an increase in blood sugar or liver glycogen levels.

3. Fasted hypophysectomized rats showed low carbohydrate levels and the administration of stilbestrol failed to increase these levels.

4. These data indicate that stilbestrol exerts its effect on carbohydrate levels, particularly liver glycogen, by stimulating the release of adrenotrophic hormone from the hypophysis. This latter factor, in turn, stimulates the production of adrenal cortical hormone, of the type characterized by an atom of oxygen on carbon 11. We believe that it is such adrenal cortical hormone which produces the effects on carbohydrate levels that have been described following the administration of stilbestrol.

REFERENCES

(1) JANES, R. G. AND W. O. NELSON. Proc. Soc. Exper. Biol. Med. **43**: 340, 1940.
(2) JANES, R. G. AND W. O. NELSON. This Journal **136**: 136, 1942.
(3) GRIFFITHS, M., H. P. MARKS AND F. G. YOUNG. Nature **147**: 359, 1941.

(4) INGLE, D. J.   Endocrinology 29: 838, 1941.
(5) JANES, R. G.   Anat. Rec. 82: 32, 1942.
(6) MERCKEL, C. AND W. O. NELSON.   Anat. Rec. 76: 391, 1940.
(7) NELSON, W. O. AND J. HICKMAN.   Proc. Soc. Exper. Biol. and Med. 36: 828, 1937.
(8) BRITTON, S. W. AND H. SILVETTE.   Cold Spring Harbor Symposia on Quantitative Biology 5: 357, 1937.
(9) LONG, C. N. H., B. KATZIN AND E. FRY.   Endocrinology 26: 309, 1940.
(10) KENDALL, E. C.   Arch. Path. 32: 474, 1941.
(11) GOOD, C. A., H. KRAMER AND M. SOMOGYI.   J. Biol. Chem. 100: 485, 1933.
(12) HOUSSAY, B. A. AND A. BIASOTTI.   Endocrinology 15: 511, 1931.

THE ESTIMATION OF RENAL FUNCTION IN THE RAT BY THE USE OF DIODRAST AND INULIN

SYDNEY M. FRIEDMAN AND CONSTANCE A. LIVINGSTONE[1]

From the Department of Anatomy, McGill University, Montreal, Canada

It has been shown in the dog (1, 2) and man (3) that assessment of renal excretory function may be made by the combined use of the foreign substances inulin and diodrast. The use of inulin as a measure of glomerular filtration has been established over a wide range of species (1, 4). In combination with inulin, the use of diodrast as a measure of effective renal blood flow (2, 5) and tubular excretory mass (5, 6) has been widely applied.

It has recently been shown that certain hormones exert morphological effects on the kidney (7). The literature on this question has been reviewed by Selye (8). Physiological effects of hormones on the kidney are suspected, if not in all cases known; for example, the rôle of the kidney in adrenal cortical disease, the effects of the posterior pituitary hormones on diuresis, of thyroxin on diuresis, and the still debated rôle of the kidney in parathyroid disturbances.

It therefore seemed of interest to try to apply, for use in the rat, the methods of renal investigation devised by Smith and co-workers for the dog, since for hormone studies the rat possesses specific advantages over the dog. After prolonged methodological study, in which numerous procedures were attempted, found unsatisfactory and discarded, the following method was evolved.

Certain major modifications which must be introduced in the application of the method to the rat are the following: *a.* A longer period for the collection of urine must be used than is necessary in larger animals. *b.* Priming and then sustaining infusions of diodrast and inulin could only be given to anesthetized rats, and for long periods this would be neither feasible nor could such animals be considered normal. The intravenous injection of inulin was attempted at first, as this substance is known to be poorly absorbed in other species (9), but it was found that the slope of disappearance from the blood varied so much from animal to animal that mid-point values gave inconstant and impossible results. Later, both inulin and diodrast were administered by subcutaneous injection, from which diodrast is well absorbed and from which inulin is also sufficiently well absorbed in the rat, particularly if one waits long enough for the peak blood value to be reached before starting the urine collection period. In this case we found the slope of fall to be more uniform and gradual, and a mid-point value more accurate and reliable. *c.* In the rat methods must allow for the obvious limitation that one cannot withdraw repeated samples of blood.

PROCEDURE. Male albino rats weighing more than 125 grams were used in all cases. At zero time diodrast to give the desired plasma concentration was

administered. We found that 0.0002 cc. of diodrast per gram body weight, for each milligram per cent of I desired would give approximately the correct level. The diodrast to be administered is made up to 4 cc. with 2 per cent sodium sulphate and injected subcutaneously. At twenty minutes, 2 cc. of 2 per cent inulin in physiological saline, warmed to body temperature, is given subcutaneously together with 1.5 cc. of inulin intraperitoneally. (The inulin is pretreated by adsorption on charcoal followed by hot filtration through a Seitz EK filter to remove pyrogen.) At sixty minutes the animal is placed in a special holder and the penis foreskin is ligated. The holder consists of a coil spring, which is applied to the dorsum, and is connected to wire nooses which are slipped over the upper jaw and hind limbs. Before ligation care must be taken that the bladder is empty.

Although the excitement caused by setting the holder in place usually causes voluntary micturition, complete emptying is ensured by manual expression of the bladder; the thumb is pressed firmly into the pelvis and then moved slowly upwards. This is repeated and the penis dried with absorbent before ligation. The ligature is a purse string suture and the knot is tied over a copper wire insert which can later be pulled to free the tie. The collection period must begin exactly at sixty minutes, hence the above manipulation is started a few minutes beforehand.

At eighty-five minutes the animal is anesthetised and 1.5 cc. of blood is withdrawn from the carotid artery, using a 24 gauge needle and heparin as an anticoagulant. The blood is immediately centrifuged. This mid-period blood must be completely drawn at ninety minutes.

At 112 minutes the animal is placed over a funnel, the ligature is removed from the urethra, and the urine collected when and as it flows until 120 minutes. Manual expression is usually used to ensure complete collection. The time from the start of collection to the time of obtaining the last drop is the collection period.

When F was to be estimated the animal was immediately anesthetised at the end of the collection period and 1.8 cc. of blood drawn from the remaining carotid artery. This blood was carefully removed from the syringe to avoid aeration, and immediately centrifuged in a capped tube. Following this the plasma was removed, 0.3 cc. was reserved for diodrast analysis and the remainder ultrafiltered for 3 hours at pCO_2 40 mm. Hg, and 37°C. The diodrast concentration in the ultrafiltrate was then determined. Small collodion bags for this purpose were made in centrifuge tubes and a small area at the bottom of the bag thus made was used.

Chemical determinations. Diodrast is determined according to the method of White and Rolfe (10) using 0.0025 N sodium thiosulphate for urine and 0.0005 N for plasma. The urine volume is noted immediately after collection and made up to 50 cc. with water, and 1 cc. aliquots of this are used in the determination, while 0.5 cc. of plasma filtrate is used in the determination of the plasma. Inulin is determined according to the method of Corcoran and Page (11) using 0.2 cc. of yeast-treated plasma filtrate and 0.2 cc. of yeast-treated urine diluted as above.

Calculations. Clearances for both diodrast and inulin are determined by the formula $C = UV/P$ and are expressed in the tables as cubic centimeter per gram body weight per minute. Clearances were calculated per unit of surface area, using Meeh's formula for the rat (12), but we found that this offers little advantage since the proportionality with body weight is as good.

The rate of tubular excretion, T, is the difference between the total excretion per minute, and the quantity excreted by filtration (5), i.e.,

$$T = UV - PIWF$$

where P is the quantity of solute in each cubic centimeter of plasma, I the concurrent rate of glomerular filtration as measured by the simultaneous inulin clearance, W the fraction of water in the plasma, and F the fraction of solute which is free in the plasma and therefore available for filtration. For the accurate determination of F the adsorption isotherm for diodrast in rat plasma should be determined and related to plasma albumin, but because of circumstances beyond our control we were unable to do this and hence approximated F by the method outlined above in a few experiments only. For all other experiments a mean value of 0.62 was used. This latter was considered valid only at the lower plasma values where a difference of ± 0.2 in F would give a maximum difference of only ± 0.0001 mgm. in T. W was determined from the formula $W = 1 - \dfrac{\text{per cent plasma protein}}{100}$. Since the average plasma protein per cent in our rats was 5.63 ± 0.43, W was 0.94. According to the accepted terminology the maximal rate of tubular excretion is designated as Tm_D (5).

RESULTS. Table 1 summarises our complete findings on a series of 31 animals. The mean value for inulin clearance is 0.0027 cc./gm.b.wt./min., with a deviation from the mean of ± 0.0006 or 22.2 per cent.

In table 2 we have summarised and analysed the data for diodrast clearance. The results are first grouped for comparison with spreads of 2 mgm. per cent being considered a unit, except for group 6 in which a spread of 3 mgm. per cent I is considered as a unit because of the scarcity of the data at this level. The analysis shows no difference between groups 1, 2 and 3, and again no difference between 4 and 5. Group 6 probably represents an intermediate between 5 and 7 since there are only 5 chances in a hundred that it is part of group 5, and less than 1 chance that it is part of group 7. Further analysis shows that on regrouping, II is significantly different from either I or III. Accordingly we can plot from these data only 3 points on the curve of diodrast clearance against plasma concentration, *a*, below 10 mgm. per cent I where effective blood flow is not measurable; *b*, a range of clearance certainly between 10 and 14 mgm. per cent I where renal blood flow is measurable, and *c*, a depressed clearance range above this level. From the data in the group with plasma levels between 10 and 14 mgm. per cent it would appear that the mean renal blood flow is

0.033 cc./gm.b.wt./min. This represents about one-third of the total circulating blood volume in the rat if 7 per cent of body weight is used to determine the value of the latter term.

TABLE 1

NUMBER	BODY WEIGHT	COLLECTION TIME	URINE VOLUME	PLASMA		INULIN CLEARANCE	DIODRAST CLEARANCE	T	F
				Inulin	Diodrast				
	grams	minutes	cc.	mgm. per cent	mgm. per cent I	cc./gm./min.	cc./gm./min.	mgm./gm./min.	
1	160	60	0.3	37.5	4.4	0.0022	0.0101	0.00039	
2	157	58	0.1	31.0	5.0	0.0026	0.0080	0.00033	
3	154	58	0.1	36.0	5.7	0.0034	0.0095	0.00043	
4	180	60	0.2	45.0	5.9	0.0029	0.0140	0.00073	
5	178	60	0.2	31.0	6.3	0.0024	0.0096	0.00049	
6	126	60	0.1	37.5	7.4	0.0028	0.0080	0.00047	
7	162	60	0.3	47.5	7.6	0.0026	0.0110	0.00073	
8	133	60	0.1	50.0	7.8	0.0037	0.0109	0.00067	
9	152	58	0.2	12.5	8.0	0.0011	0.0119	0.00090	
10	150	60	0.5	40.0	8.9	0.0028	0.0112	0.00088	0.50
11	181	60	0.2	31.0	9.1	0.0044	0.0088	0.00059	
12	155	60	0.1	40.0	9.5	0.0016	0.0079	0.00065	
13	176	58	0.5	25.0	10.1	0.0027	0.0171	0.00158	
14	180	55	0.2	35.0	10.1	0.0017	0.0111	0.00100	0.76
15	135	58	0.2	52.5	10.8	0.0025	0.0120	0.00116	
16	185	60	0.5	30.0	10.8	0.0024	0.0142	0.00133	
17	195	60	0.6	42.5	10.9	0.0023	0.0120	0.00117	0.66
18	200	60	0.6	42.5	11.2	0.0031	0.0133	0.00133	0.50
19	200	60	0.7	37.5	11.5	0.0031	0.0160	0.00169	0.46
20	178	58	0.4	25.0	13.0	0.0034	0.0149	0.00160	0.69
21	205	55	0.4	26.0	13.3	0.0032	0.0114	0.00131	0.53
22	195	60	0.5	25.0	14.6	0.0024	0.0143	0.00188	0.64
23	185	63	0.5	31.0	16.1	0.0026	0.0104	0.00146	0.71
24	176	51	0.6	36.0	17.8	0.0027	0.0117	0.00168	
25	175	60	0.5	35.0	18.8	0.0032	0.0109	0.00156	
26	192	62	0.5	33.3	23.3	0.0022	0.0070	0.00127	0.77
27	200	60	0.5	30.0	23.3	0.0025	0.0063	0.00108	0.70
28	165	65	0.6	20.0	25.4	0.0036	0.0096		
29	160	60	0.4	50.0	30.7	0.0035	0.0070		
30	150	70	0.4	42.5	33.9	0.0033	0.0069		
31	180	58	0.4	25.0	35.6	0.0032	0.0061	0.00169	0.50
Mean..						0.0027			0.62
σ..						±0.0006			

Similarly we have summarised and analysed the data for T as above. Again there was no statistical difference between groups 1, 2, 3 on the one hand and groups 4, 5, 6 and 7 on the other, while these two groups taken together showed that above 10 mgm. per cent I the tubular excretion is significantly higher than

below this level.   The average of the data for T obtained from animals with plasma levels above 10 mgm. per cent I is 0.00142 mgm./gm. b.wt./min., while σ is ±0.00024 or 17.6 per cent.   On this basis it appears that above this plasma

TABLE 2

PLASMA DIODRAST	CLEAR-ANCE	GROUP	MEAN	PROBABILITY BETWEEN GROUPS	GROUP	PROBABILITY BETWEEN GROUPS	MEAN	σ	$\frac{\sigma}{X} \times 100$
	cc./gm./ min.								
4.4	0.0101								
5.0	0.0080	1	0.0104	1–2 p>0.05					
5.7	0.0095			1–3 p>0.05					
5.9	0.0140			1–4 p<0.05					
6.3	0.0096			2–3 p>0.05					
7.4	0.0080	2	0.0099	2–4 p<0.05	I		0.0101		
7.6	0.0110								
7.8	0.0109								
8.0	0.0119			3–4 p<0.01					
8.9	0.0112	3	0.0100						
9.1	0.0088								
9.5	0.0079								
10.1	0.0171			4–5 p>0.05					
10.1	0.0111			4–6 p<0.05					
10.8	0.0120	4	0.0137			II–I p<0.01			
10.8	0.0142					II–III p< 0.01			
10.9	0.0120				II		0.0133	±0.0021	15.8
11.2	0.0133								
11.5	0.0160								
13.0	0.0114			5–6 p<0.05					
13.3	0.0114	5	0.0123						
14.6	0.0143								
16.1	0.0104								
17.8	0.0117	6	0.0110	6–7 p<0.01					
18.8	0.0109								
23.3	0.0070								
23.3	0.0063								
25.4	0.0096	7	0.0071		III		0.0090		
30.7	0.0070								
33.9	0.0069								
35.6	0.0061								

level the calculation yields Tm_D.   The data computed according to surface area yield an average maximum clearance for diodrast of 78.7 cc./min./M^2 for plasma and Tm_D of 8.4 mgm./min./M^2.

SUMMARY

A method for estimating renal function in the rat by the simultaneous use of diodrast and inulin is presented.

Data for the normal rat indicate an average inulin plasma clearance of 0.0027 cc./gm. body weight/min., diodrast plasma clearance of 0.0133 cc./gm./min., Tm_D of 0.00142 mgm./gm./min.; C_D/Tm_D averages 9.3, and C_{IN}/C_D is 0.20 (or as Filtration Fraction 20.02 per cent).

REFERENCES

(1) SHANNON, J. A. This Journal **112**: 405, 1935.
(2) ELSOM, K. A., P. A. BOTT AND E. H. SHIELS. This Journal **115**: 548, 1936.
(3) GOLDRING, W., H. CHASIS, H. A. RANGES AND H. W. SMITH. J. Clin. Investigation **19**: 739, 1940.
(4) SHANNON, J. A. J. Cell. and Comp. Physiol. **5**: 301, 1934.
(5) SMITH, H. W., W. GOLDRING AND H. CHASIS. J. Clin. Investigation **17**: 263, 1938.
(6) WHITE, H. L. AND P. HEINBECKER. Proc. Soc. Exper. Biol. and Med. **43**: 8, 1940.
(7) SELYE, H. J. Urol. **42**: 637, 1939.
(8) SELYE, H. J. Urol. **46**: 110, 1941.
(9) SMITH, H. W. Physiology of the kidney. Oxford Univ. Press, 1937.
(10) WHITE, H. L. AND D. ROLFE. Proc. Soc. Exper. Biol. and Med. **45**: 433, 1940.
(11) CORCORAN, A. C. AND I. H. PAGE. J. Biol. Chem. **127**: 601, 1939.
(12) LEE, M. O. This Journal **89**: 24, 1929.

THE INABILITY OF PURIFIED OR CRUDE KIDNEY EXTRACT (RENIN) TO REDUCE THE BLOOD PRESSURES OF HYPERTENSIVE DOGS[1]

MEYER FRIEDMAN, H. E. KRUGER AND A. KAPLAN

From The Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital, San Francisco, California

In 1941, Wakerlin and Johnson (1) reported a fall in blood pressure occurring in four hypertensive dogs following daily intramuscular injections of hog renin over a period of several months. The serums of these treated dogs neutralized the acute pressor effect of renin in assay animals. More recently these authors (2) have reported that preliminary injections of renal extracts into dogs whose renal arteries were subsequently constricted by means of clamps, appeared to diminish the degree of hypertension that usually follows this type of kidney manipulation. Other workers (3), however, employing crude and purified renin solutions prepared in a different manner from those of Wakerlin and Johnson, failed to confirm their findings. In view of the conflicting results concerning the efficacy of daily renin therapy in hypertension, it was deemed advisable in this laboratory to repeat the experiments of Wakerlin and his co-workers as closely as possible, using *a*, a kidney extract prepared according to their directions, and *b*, a purified renin extract.

METHODS. The method of producing hypertension in the majority of the animals employed in the study was similar to the technic of the Wakerlin group and has been described previously (4).

Seven hypertensive dogs were subjected to daily injections of kidney extract for a period of three to four months. Three of them (dogs 13, 28 and 30) received by intramuscular injection purified renin (fraction D) (5), and the remaining four (dogs 5, 27, 29 and 32) received by the subcutaneous and intramuscular route a relatively crude renin prepared according to the directions of Wakerlin and Johnson (6), hereafter referred to as W-J renin. The daily dosage was equivalent to one gram of fresh kidney cortex per kilogram of body weight.

The ability of the plasma of injected animals to neutralize the pressor effect of renin was measured according to the method of Wakerlin and Johnson (1).

RESULTS AND DISCUSSION. The prolonged injection of purified renin or W-J renin into seven hypertensive dogs was not followed by any significant change in their blood pressures (table 1) or in the ability of their blood plasmas to neutralize the pressor effect of renin (table 2). As indicated in table 2, there was considerable monthly variation in the pressor effect of a standard renin solution, after a preliminary 24 hour refrigeration with the blood plasma of these hypertensive dogs. Whether this variation was due to erratic responses of different test dogs or to fluctuations in the antirenin content of their plasmas is not known.

TABLE 1

The effect of daily injections of renin upon the blood pressure of hypertensive dogs

DOG NO.	DURATION OF HYPER-TENSION BEFORE INJECTIONS	DURATION OF INJECTIONS	AVERAGE ARTERIAL BLOOD PRESSURE							CHANGE IN BLOOD PRESSURE††
			Before renin injections		During renin injections					
			Before* hyper-tension	After** hyper-tension	1st to 4th week†	5th to 8th week†	9th to 12th week†	13th to 16th week†		
					Receiving purified renin					
	days	days	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg		per cent
13	90	123	112	176	165	168	162	167		−5.0
28	67	123	110	173	161	150	159	160		−7.5
30‡	54	74	124	163	176	190	226			+38.5
					Receiving W-J renin					
5	115	114	113	181	196	193	192	178		−2.0
27	113	119	138	188	190	191	185	199		+6.0
29	102	119	128	167	166	164	167	184		+10.8
32	95	96	134	172	173	179	183			+6.5

* Average blood pressure of the week preceding the kidney operation.

** Average blood pressure of the week preceding the start of daily renin injections.

† Average blood pressure during the four week interval.

‡ Died of uremia 10.5 weeks after daily renin injections were instituted.

†† Per cent change of last average blood pressure from average hypertensive pressure before renin injections were started.

TABLE 2

Pressor effect of a standard renin solution when injected into anesthetized dogs following its refrigeration* with plasma of hypertensive dogs receiving daily injections of renin

PLASMA USED DOG NO.	MAXIMUM PRESSOR RESPONSE OF TEST DOGS** TO REFRIGERATED RENIN-PLASMA MIXTURE					
	Before renin injections†	During renin injections				
		End of 4th week	End of 6th week	End of 8th week	End of 12th week	End of 16th week
			Receiving purified renin			
		mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg
13	40		30		54	40
28	37		30		28	26
30‡	32		28			
			Receiving W-J renin			
5	83	54		68	26	42
27	53	40		90	40	38
29	38	42		36	69	62
32	24	36		76	22	

* Two volumes of plasma were refrigerated with one volume of renin. For assay purposes, each normal dog received the equivalent of 0.5 grams kidney cortex per kilo body weight.

** Anesthetized, nephrectomized dogs used as test animals for purified renin group; anesthetized, normal dogs used as test animals for W-J renin group.

† One day prior to administration of renin.

‡ Died of uremia 10.5 weeks after daily renin injections were instituted.

The plasmas of dogs 29 and 32 showed no increase whatever in their ability to neutralize the vasopressor effect of renin, while dogs 5 and 27 showed some increase in antirenin content at the end of the third and fourth months.

Although a reduction in the blood pressure of hypertensive dogs could not be obtained in our laboratory by means of the prolonged injection of renal extracts containing a high potency of renin, there are many reports concerning the reduction in blood pressure by the administration of other types of kidney extracts (7, 8, 9), by the subcutaneous transplantation of kidney tissue (10), and by the injection of tyrosinase solutions obtained from mushrooms (11). While there may be some specific depressant at work in all of these experiments, there is also the strong possibility that the effect is due to a non-specific factor. In this last connection, it may be mentioned that whereas recently we have obtained a reduction in the blood pressure of several hypertensive dogs following the administration of a kidney extract prepared according to the method given by Page and associates (8), this reduction was usually accompanied by toxic reactions which in severe cases appeared very similar to actual left ventricular failure (rapid respiration, tachycardia, pulmonary edema). Severe untoward symptoms were noted also by Williams et al. (7) following the reduction in hypertension induced by the parenteral administration of renal extracts. So far, there has been little evidence presented as to the mechanism whereby these extracts are effective.

SUMMARY

The daily injection of purified and W-J renin into seven hypertensive dogs for a period of three to four months was not accompanied by a significant reduction in blood pressure. In the majority of the dogs, also, there was no increase in the ability of their plasma to neutralize the pressor effect of renin.

REFERENCES

(1) WAKERLIN, G. F. AND C. A. JOHNSON. J. A. M. A. 117: 416, 1941.
(2) WAKERLIN, G. F., C. A. JOHNSON, E. L. SMITH, J. R. WEIR AND G. A. OLANDER. Federation Proc. I: Part II 88, 1942.
(3) WINTERNITZ, M. C., E. MYLON AND R. KATZENSTEIN. Yale J. Biol. and Med. 13: 789, 1941.
(4) FRIEDMAN, M., H. E. KRUGER AND A. KAPLAN. Proc. Soc. Exper. Biol. and Med. 50: 56, 1942.
(5) HELMER, O. M. AND I. H. PAGE. J. Biol. Chem. 127: 757, 1939.
(6) WAKERLIN, G. F. AND C. A. JOHNSON. Private communication.
(7) WILLIAMS, J. R., A. GROLLMAN AND T. R. HARRISON. This Journal 130: 496, 1940.
(8) PAGE, I. H., O. M. HELMER, K. G. KOHLSTAEDT, P. J. FOUTS AND G. F. KEMPF. J. Exper. Med. 73: 7, 1941.
(9) JENSEN, H., W. C. CORWIN, S. TOLKSDORF, J. J. CASEY AND F. BAMMAN. J. Pharmacol. and Exper. Therap. 73: 38, 1941.
(10) RODBARD, S., L. N. KATZ AND M. SOKOLOW. Proc. Soc. Exper. Biol. and Med. 44: 360, 1940.
(11) SCHROEDER, H. A. AND M. H. ADAMS. J. Exper. Med. 73: 531, 1941.

THE EFFECT OF THIAMINE HYDROCHLORIDE ON THE ENERGY VALUE OF DEXTROSE STUDIED IN RATS BY THE SINGLE FOOD CHOICE METHOD[1]

CURT P. RICHTER AND KATHERINE K. RICE

From the Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital

In previous papers we reported the results of experiments in which a single carbohydrate, fat or protein constituted the sole source of nourishment for rats (Richter, Holt and Barelare, 1938; Richter, 1941). Survival time and spontaneous activity were used to measure the nutritional value of common representatives of each of the three main classes of foodstuffs. The rats lived longer on dextrose (57 days) than on any of 6 other carbohydrates; longer on saltless butter (53 days) than on any of 7 other fats; and longer on a casein digest (47 days) than on any of 4 other proteins.

In the following experiments dextrose was selected for a more detailed study on larger groups of animals using definitely improved single food choice methods. Studies were also made of the effect produced on survival time and energy output of these dextrose rats by giving them access to thiamine hydrochloride. In a preliminary study Holt and Kajdi (1939) reported that access to this vitamin prolonged the life of rats kept on a single food dextrose diet. They did not measure activity or any other effects. We have repeated these experiments, and have also included a comparison of hydrated with anhydrous dextrose. Records were made of the daily intake of the dextrose, thiamine hydrochloride, and water; also of the effects produced by this diet on the vaginal smears and body-weight.

METHODS. The individual all-metal cages used for these experiments consisted of a living compartment 11 x 3½ x 5 inches, made of ⅛ inch mesh wire cloth, and of a ¼ inch mesh revolving drum 12½ inches in diameter and 6 inches wide, with a cyclometer attached to the axle to record revolutions of the drum in either direction. The living compartment contained a food cup designed to eliminate spillage and two inverted graduated 100 cc. bottles. At least once every 10 days the drums and cyclometers were tested by recording the number of revolutions of the drum produced by a standard impetus (the force exerted by the fall of a 675 gram weight as it turns a 3¾ inch pulley attached to the end of the axle of the drum through 270°; each drum was set to turn at least 60 revolutions under these conditions). This test made certain that all of the drums turned with essentially the same ease, and that the cyclometers worked freely to give an accurate record of the running activity.

In almost all instances the rats deposit their feces in the revolving drum rather than in the living compartment. As the drum revolves the feces quickly reach the ¼ inch space between the drum and the central partition and fall through to

the pan supported several inches below and beyond the reach of the rats. Since the completion of the previous experiments, it has been discovered that the wooden disc drums that were then in use did not in all instances prevent the rats from reaching their feces. Warping of the wooden disc made the space between the edge of the running drum and the central partition wider on one side of the drum than on the other, thus making it possible for the rats to reach through to the pan several inches below to pick up their feces. This coprophagy probably accounts for the longer survival time of the rats in the previous than in the present experiments.[2] The consistency of the results of the present experiments suggests that this defect has been remedied.

The rats, aged 41 to 58 days, were placed in the activity cages, and given free access to tap water and the standard stock diet made according to the following formula: graham flour, 72.5 per cent; skim milk powder, 10.0 per cent; casein, 10.0 per cent; butter, 5.0 per cent; calcium carbonate, 1.5 per cent; sodium chloride, 1.0 per cent.

Approximately 15 days later, when they had reached an average weight of 140 grams (119 to 163 grams) and showed regular 4 to 5 day vaginal smear cycles, they were divided into four groups and started on the single food choice. That is, they were given free access to tap water and either (1) anhydrous dextrose, or (2) dextrose hydrate, or (3) anhydrous dextrose and a 0.02 per cent solution of thiamine hydrochloride, or (4) dextrose hydrate and a 0.02 per cent solution of thiamine hydrochloride.[3]

Records were made daily of the running activity, vaginal smears, and of the intake of the stock diet or dextrose, thiamine hydrochloride solution and water. Body-weight was recorded weekly. The thiamine hydrochloride solution was made fresh weekly and changed in the bottles twice weekly. All of the rats were thoroughly inspected for signs of deficiency at least once each week. A total of 57 female rats were used in these experiments: 24 on anhydrous dextrose; 9 on dextrose hydrate; 16 on anhydrous dextrose and thiamine hydrochloride; and 8 on dextrose hydrate and thiamine hydrochloride.

In order to be able to make comparisons between the effects produced on activity by these different diets we used as far as possible only rats whose average daily running activity had reached a level above 9,000 revolutions per day at the end of the 15 day preliminary period. However, on account of the relatively small size of our rat colony and the limited number of activity cages we were forced to use some animals which did not attain this level of activity.

---

[2] After the experiments herein reported were completed it was decided to test the effect of unlimited coprophagy in animals on a dextrose diet. Accordingly three rats were started on the single food dextrose and tap water regime and were also given access, in a separate food cup, to their total daily feces. These animals lived 54, 54, and 55 days respectively, thus strongly indicating that the ability to reach their feces had been responsible for the longer survival time of the animals in the previous than in the present experiments.

[3] The Corn Products Company kindly furnished the anhydrous dextrose (special anhydrous) and the dextrose hydrate (cerelose—9 per cent water). Merck and Company kindly supplied the crystalline thiamine hydrochloride.

RESULTS.   Figures 1 A and B show records of two typical rats, one with access only to dextrose hydrate and tap water, the other with access to dextrose hydrate and tap water and a 0.02 per cent solution of thiamine hydrochloride.   The ordinates show activity in number of revolutions of the drum, body-weight and food intake in grams, and intake of thiamine hydrochloride in cubic centimeters; the abscissae show age in days.   During the 15-day period on the stock diet the activity of the rat on dextrose alone increased from approximately 4,000 revolutions per day to a level near 18,000 (from 2.5 to 11.2 miles).   During the first 20
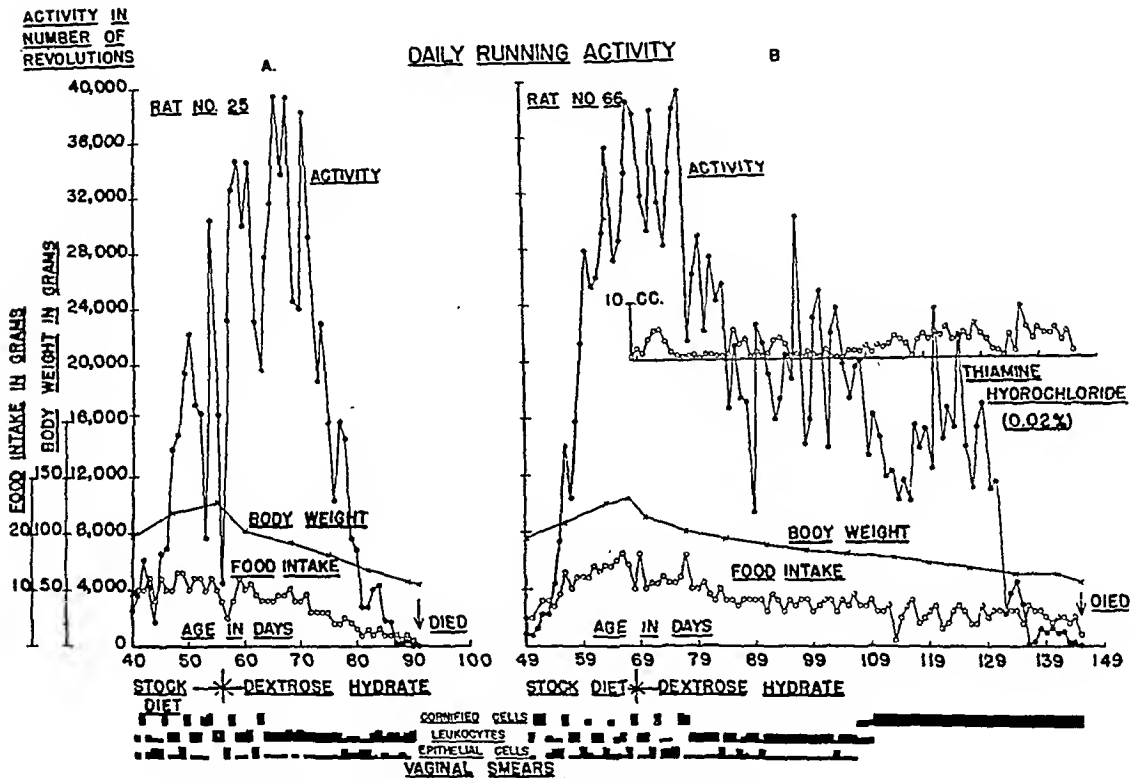
Fig. 1. Data from two typical rats: A, with access to dextrose hydrate and tap water only. B, with access to dextrose hydrate, tap water, and thiamine hydrochloride (0.02 per cent).

days on the diet restricted to dextrose hydrate the rat became very active, on two days reaching peaks near 40,000 revolutions per day (25 miles).   After the 20th day the activity dropped off markedly and reached a level of only a few hundred revolutions during the last few days that the rat lived (37 days).   On the dextrose diet body-weight decreased at a rapid and steady rate.   The dextrose intake did not begin to show a rapid decrease until after the 15th day.   Vaginal smears (shown at the bottom of the chart) showed the regular 4–5 day cycles of cornified cells for the period on the stock diet and for 6 days on the dextrose diet. After that the smears showed only a constant picture of leukocytes and epithelial cells.   The rat which had access to a 0.02 per cent solution of thiamine hydro-

chloride in addition to the dextrose was also very active for the first 20 days on the restricted diet; after that, unlike the rat on dextrose alone, it remained quite active (with daily averages of 14,000 revolutions) up to within 15 days of death on the 78th day of this diet. Body-weight decreased much more slowly than that of the rat on dextrose alone. After the first 10 days the dextrose intake decreased slowly from 8.9 to 4.5. The intake of thiamine hydrochloride was high during the first few days, then decreased to a flat low level at which it remained for 25 days, later to increase again at a steady rate up to the next to last day of life. On the dextrose diet the vaginal smears showed two 4-day cycles

TABLE 1

DEXTROSE	NUMBER OF RATS	SEX	AVERAGE AGE AT START	AVERAGE WEIGHT AT START	SURVIVAL TIME	AVERAGE SURVIVAL TIME
			days	grams	days	days
Anhydrous	24	♀	64 (56–71)*	138 (120–157)*	27, 28, 29, 32, 33, 33, 34, 35, 36, 36, 36, 37, 37, 38, 39, 39, 40, 40, 40, 40, 41, 42, 42, 54	37
Hydrated	9	♀	62 (56–66)	148 (123–160)	32, 33, 34, 34, 35, 36, 37, 39, 42	36
Anhydrous + vitamin B$_1$	16†	♀	63 (58–73)	144 (130–163)	62, 65, 67, 67, 72, 73, 74, 74, 75, 76, 76, 78, 83, 87, 87	74
Hydrated + vitamin B$_1$	8	♀	64 (63–68)	139 (128–150)	66, 70, 72, 74, 78, 80, 81, 86	76
Control rats on no food	11	♀	57 (40–72)	141 (118–210)	3, 4, 4, 4, 4, 4, 4, 4, 4, 5, 6	4

* The numbers in parentheses show the variations in age and weight.

† The 16th rat in this group was not included in the average survival time because it escaped from its cage for some hours on the 78th day of the experiment. It was killed on the 87th day, when it appeared to be moribund.

of cornified cells and then a constant dioestrous picture of leukocytes and epithelial cells for the next 30 days. After that until the rat died the smears showed only cornified cells.

Survival times. Table 1 summarizes the results. On the anhydrous dextrose the survival time of the 24 rats averaged 37 days (27–54, with only one animal living longer than 42 days). On the hydrated dextrose the survival time of 9 rats had almost the same average—36 days (32–42). Thus the hydrated and anhydrous forms of dextrose kept the rats alive for almost exactly the same length of time.

When given access to a 0.02 per cent solution of thiamine hydrochloride 15

rats[4] on anhydrous dextrose survived an average of 74 days (62-87), while 8 rats on the hydrated dextrose with access to the thiamine hydrochloride solution lived an average of 76 days (66-86); that is, the addition of the vitamin supplement almost exactly doubled the survival time of rats on a single food dextrose diet.

Body-weight. Figure 2 shows .the body-weight curves for the four groups of rats. The two groups on anhydrous and hydrated dextrose (1 and 2) had almost identical curves, decreasing at a steady rate from approximately 140 grams on the first day of the single food diet to an average of 64 grams at death. The other two groups on anhydrous and hydrated dextrose (3 and 4), which had access to the vitamin supplement, also showed extremely little variation; however, their curves deviated markedly from those of the two groups of rats on
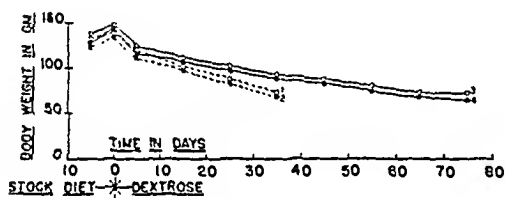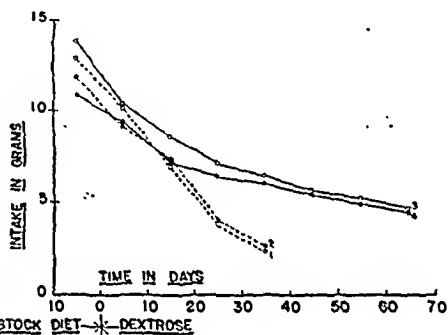
Fig. 2   Fig. 3

Fig. 2. Graph showing average body-weights of: 1, 24 rats with access to anhydrous dextrose and tap water; 2, 9 rats with access to dextrose hydrate and tap water; 3, 16 rats with access to anhydrous dextrose, tap water, and thiamine hydrochloride (0.02 per cent); 4, 8 rats with access to dextrose hydrate, tap water, and thiamine hydrochloride (0.02 per cent).

Fig. 3. Food intake of: 1, 24 rats with access to anhydrous dextrose and tap water; 2, 9 rats with access to dextrose hydrate and tap water; 3, 16 rats with access to anhydrous dextrose, tap water, and thiamine hydrochloride (0.02 per cent); 4, 8 rats with access to dextrose hydrate, tap water, and thiamine hydrochloride (0.02 per cent).

dextrose without the vitamin, in that they showed a far less rapid decrease. At the end of 65 days these rats weighed somewhat more than the other rats did after 35 days, but at death all four groups reached approximately the same weight (average—62 grams).

Intake of dextrose and thiamine hydrochloride. Figure 3 shows the dextrose intake curves of all four groups in 10-day averages. Here again the rats on anhydrous and hydrated dextrose without access to thiamine hydrochloride (1 and 2) had almost identical curves. During the first 10-day experimental period the dextrose intake for the two groups averaged approximately 9.5 grams, which is

[4] The 16th rat in this group was *not* included in the average survival time because it escaped from its cage for some hours on the 78th day of the experiment. It was killed on the 87th day, when it appeared to be moribund.

only about 2.5 grams less than the intake of stock food in the preceding 10 days. However, during the second, third, and fourth 10-day periods the intake dropped steadily from 10 to 7 to 4 to 2.5 grams, with an average intake of 1.8 grams on the day preceding death.

The animals on anhydrous and hydrated dextrose with access to thiamine hydrochloride (3 and 4) also had almost identical intake curves, starting with an average daily intake of approximately 10 grams during the first 10-day period. Their intake decreased, however, at a much slower rate than that of the rats on dextrose alone. For the last 10 days of life their averages were almost twice as high as for the rats on dextrose alone.

The intake curves of the thiamine hydrochloride are quite similar in the two groups receiving this supplement (fig. 4A). All the rats showed a marked appe-
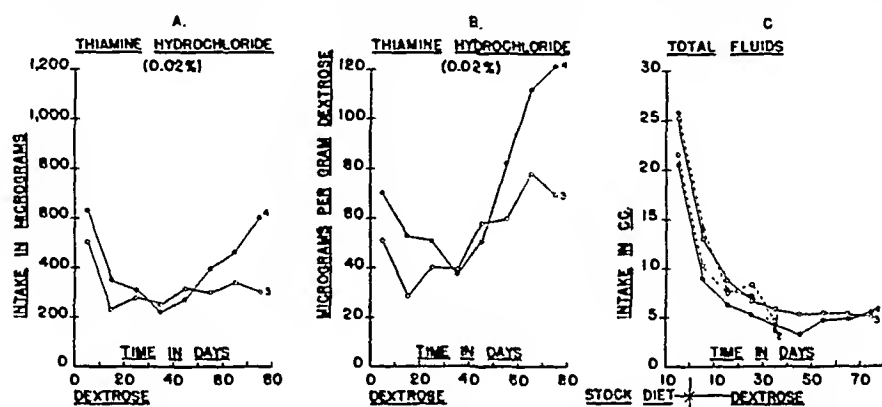
Fig. 4A. Intake of thiamine hydrochloride in micrograms.

　　　　B. Intake of thiamine hydrochloride in micrograms per gram dextrose—for: 3, 16 rats with access to anhydrous dextrose, tap water, and thiamine hydrochloride (0.02 per cent); 4, 8 rats with access to dextrose hydrate, tap water, and thiamine hydrochloride (0.02 per cent).

　　　　C. Total fluid intake (water or water plus vitamin solution) for groups 3 and 4 above and for: 1, 24 rats with access only to anhydrous dextrose and tap water; 2, 9 rats with access only to dextrose hydrate and tap water.

tite for the vitamin during the first few days, followed by an abrupt decrease in intake. A much lower level was maintained during the next several weeks, while in the second half of the experimental period the intake was again increased, in some cases very markedly. When the thiamine hydrochloride consumption is calculated in terms of micrograms per gram of dextrose, the rise during the later weeks becomes very much more striking, as shown in figure 4B. This increased need manifested itself about the 30th to 40th day; that is, about the time that it was found that the rats on the diet restricted to dextrose died.

Fluid intake. Figure 4C shows the total fluid intake of all four groups of rats. No corrections have been made, either for evaporation, which varies from 0.6 to 1 cc. per day, or for the water content of the dextrose hydrate (9 per cent). All four curves are essentially similar, with an abrupt drop in daily fluid consumption from 20-25 cc. to 10-15 cc. when the animals are first changed from the

stock food to the experimental diet, followed by a slower decrease to an intake of between 4 and 6 cc. in the 30 to 40 day period. The two groups receiving the thiamine supplement lived another 4 to 5 weeks, maintaining approximately this same level of fluid intake. In contrast with the food intake all four groups showed essentially the same fluid intake at the time of death.

Activity. Figure 5 summarizes the results giving the records of all of the rats from each group that had a daily average above 9,000 revolutions for the last 10-day period on the stock diet. Sixteen rats on anhydrous dextrose and 9 rats
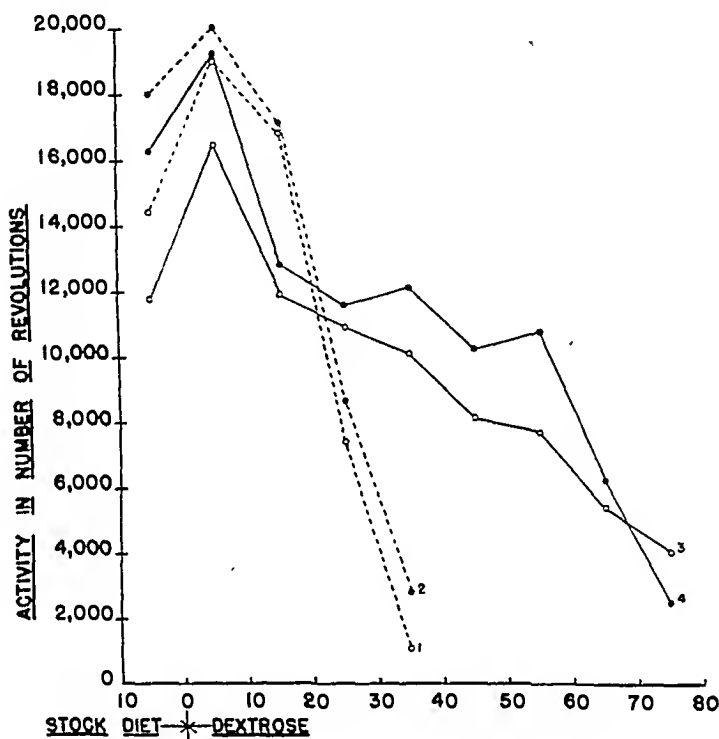
Fig. 5. Activity of 1, 16 rats with access to anhydrous dextrose and tap water; 2, 9 rats with access to dextrose hydrate and tap water; 3, 10 rats with access to anhydrous dextrose, tap water, and thiamine hydrochloride solution; 4, 3 rats with access to dextrose hydrate, tap water, and thiamine hydrochloride solution (0.02 per cent).

Only those animals averaging more than 9000 revolutions per day during the last 10 days on stock food are included.

on dextrose hydrate had almost identical curves throughout the experimental period (1 and 2). During the first and second 10-day periods on the dextrose diets the daily activity averaged higher than for the last 10 days on the stock diet, while in the two following 10-day periods it dropped off very sharply. During the last few days before death most of the rats averaged less than a hundred revolutions per day.

Both groups of rats which received thiamine maintained a high level of activity for 50 to 60 days and in some cases were still very active one or two days before death. Figure 5 includes the running activity in 10-day averages for 10

rats on the anhydrous dextrose plus thiamine hydrochloride solution (3) and for 3 rats on dextrose hydrate plus the vitamin (4); (the other animals in these groups were not sufficiently active while on stock food to be included in the averages).

Deficiency symptoms. All of the rats showed regular 4 to 5 day vaginal smear cycles while on the stock diet, and nearly all of them completed either one or two more cycles after the start of the experimental diets. But after the first 10 days all animals on dextrose, both with and without access to the thiamine solution, showed a constant dioestrous condition. This was maintained to the time of death in the rats receiving dextrose only, while those with access to thiamine began to show a constant cornification of the vaginal epithelium after an average of 51 days (42-71), thus indicating the development of a vitamin A deficiency (Evans, 1928).

With the exception of the constant dioestrous condition of the vaginal mucosa, the animals on dextrose alone showed no specific deficiency symptoms. They were without exception markedly emaciated, with an average weight loss of approximately 50 per cent before death, but the coat, skin and eyes were in surprisingly good condition throughout the experimental period.

On the other hand, the 23 animals with access to thiamine hydrochloride solution, living twice as long, showed definite signs of deficiency in nearly all cases during the last 20 to 30 days. In addition to the constant cornification of the vaginal smears shown by all of them, twenty showed some signs of keratitis, and most of the rats showed coarsening of the hair.

DISCUSSION. On the basis of results of previous self-selection experiments it would seem very likely that in the present experiment the rats made almost the best possible use of the available dextrose; or in other words they ate just as much dextrose as they were able to utilize. On the dextrose alone they kept themselves alive an average period of 37 days, which is 33 days longer than rats live when they have no food at all, only water. Noteworthy is the fact that these rats had no characteristic signs of any nutritional deficiency. When given access to thiamine hydrochloride the rats showed by their increased dextrose intake and prolonged activity, as well as by their doubled survival time, that they were able to utilize more of the carbohydrate.

These experiments furnish further evidence, under extremely simple conditions, of the dependence of dextrose utilization on the availability of thiamine hydrochloride. The significance of the higher intake of thiamine during the second half of the experimental period is not clear at the present time.

The results of these experiments suggest the possible usefulness of the single food-choice method not only for the study of the nutritional value of individual food-stuffs but also for the study of the rôles played by the various vitamins in their utilization. This method may be applied not only to the other sugars, and to the other members of the vitamin B complex, but also to the various fats and proteins.

SUMMARY

1. Thirty-three young female rats maintained on a single food diet of dextrose and tap water survived an average of 36 days, whereas the controls given water but no food died in 4 days.

2. The dextrose rats remained extremely active for the first 20 days on the diet but were almost totally inactive during the last week of life.

3. Body-weight, food intake and water intake decreased steadily throughout the experiment.

4. These rats showed no signs of specific nutritional deficiency, but were in a constant state of diestrus after the first 10 days on the diet as evidenced by vaginal smears, and became extremely emaciated before death.

5. Twenty-four comparable rats given access to a 0.02 per cent solution of thiamine hydrochloride, in addition to the dextrose and tap water, survived more than twice as long—74 days on the average.

6. In this group activity was maintained at a good level for more than 50 days.

7. Body-weight decreased slowly, to arrive at almost exactly the same terminal weight as in the unsupplemented groups, whereas the food intake during the last 10 days of life was twice that of the dextrose animals.

8. The intake of thiamine hydrochloride calculated in micrograms per gram of dextrose fell from an average of 60 during the first 10 days to 40 in the 30–40 day period and then increased steadily to nearly 100 in the 70–80 day period.

9. The rats receiving the thiamine supplement showed definite signs of vitamin A deficiency with constant cornification of the vaginal smears after about the 50th day in all cases and development of keratitis in 20 of the 24 rats. These animals also were extremely emaciated before death.

Since this paper was submitted for publication four rats were given access to dextrose, tap water and a 0.03 per cent solution of cocarboxylase, the phosphorylation product of thiamine. These animals lived an average of 77 days—not significantly varying from the 74 days on the thiamine solution—and they too showed a marked increase in the vitamin/dextrose ratio during the latter half of the experimental period.

REFERENCES

RICHTER, C. P., L. E. HOLT, JR. AND B. BARELARE, JR.   This Journal 122: 734, 1938.
RICHTER, C. P.   This Journal 133: 29, 1941.
HOLT, L. E., JR. AND C. N. KAJDI.   Am. J. Dis. Child. 58: 669, 1939.
EVANS, H. M.   J. Biol. Chem. 77: 651, 1928.

THE IMPORTANCE OF THE THYROID IN MAINTAINING AN ADEQUATE PRODUCTION OF HEAT DURING EXPOSURE TO COLD

GORDON C. RING

From the Department of Physiology, The Ohio State University, Columbus

It is well known that there is increased activity of the thyroid during prolonged exposure to cold (see Ring, 1939, for references) and that thyroidectomized animals do not survive as well as normal animals under such conditions. Yet cold does not bring about a large increase in activity of the thyroid since a sojourn of three weeks in a refrigerator at 2 to 4 degrees centigrade increases the basal metabolism of rats only 10 or 15 per cent. The total metabolism while the animals are in the refrigerator must be about three times the basal level (see Benedict and MacLeod, 1929, and Swift and Forbes, 1939). Therefore the rise in basal metabolism due to increased thyroid activity accounts for no more than 3 to 5 per cent of the total output of heat required by the rat under such severe conditions. These facts have caused us to wonder whether the direct effect upon basal metabolism might really be of minor importance. It is possible that the greatest value of the thyroid lies in sensitizing the response to epinephrine (Goetsch effect). To test this possibility, the calorigenic response to epinephrine under varying conditions of thyroid activity has been measured. This included a study of the response to epinephrine after prolonged exposure to cold. In the course of these observations, it was found that the maximal metabolic response to cold as well as the response to epinephrine was potentiated by thyroxin.

METHOD. Male rats weighing about 200 grams were selected for these experiments. The basal metabolism was determined by using the apparatus previously described (Ring, 1940). The oxygen consumption was recorded during the four hours prior to the injection of the gelatin preparation of epinephrine[1] and for six hours thereafter. Periods of quiet were selected for calculating the oxygen consumption. A few measurements of the respiratory quotients using the Haldane principle have been made to be sure that the caloric expenditure may be satisfactorily estimated by determining oxygen consumption alone.

In studying the response following prolonged exposure to cold, rats were kept in individual cages in the refrigerator for three weeks. After they were returned to normal room temperature, a number of the rats were given ordinary drinking water but some were given water containing NaI (0.75 mgm. per cc.) in order to bring about a more rapid return of the basal metabolism to normal (see Ring, 1941). At weekly intervals, the basal metabolism and the calorigenic response to epinephrine were measured. These responses were compared with those obtained in normal rats, thyroidectomized rats and rats injected with thyroxin.

---

[1] Armour's Suprarenalin-Gelatin Mixture 1:500.

The last part of the project was to measure the maximal catabolic response to cold. Jars containing one rat each were placed in a water bath maintained at 2.4 degrees centigrade and the oxygen consumption was measured over a period of three hours or more. Most of the rats did not produce enough heat to maintain body temperature and it could be assumed that the response to cold produced the maximal prolonged elevation in metabolism of which the animal was capable. This assumption does not hold true if measurements are made after the body temperature has fallen more than two or three degrees. Under such circumstances, the stimulation of metabolism is not maximal (see Ring, 1938) and the results were not considered in this study. Some of the rats were, however, able to maintain a normal body temperature under the above conditions. It therefore became necessary to remove part of the hair from these animals if one was to be certain that the maximal catabolic stimulation had been produced. Using this method, the effects of thyroxin, thyroidectomy and removal of the adrenal medulla upon the maximal response to cold have been measured.

RESULTS. To make sure that epinephrine did not markedly affect the proportion of foodstuffs burned, respiratory quotients were determined in a few cases. The control measurements averaged 0.727 and during the six hours after receiving epinephrine these were 0.723.

In order to determine the most suitable amount of epinephrine for these experiments, the effects of injecting doses of 0.2 to 0.05 cc. were first investigated. The larger doses frequently proved fatal and so were discontinued but it was interesting to find that these caused no greater metabolic response during the first two hours than the smaller ones. They did, however, have a more prolonged effect. The increase in metabolism during the first two hours after the injection of epinephrine averaged for 0.20 cc. 52.5 per cent, for 0.15 cc. 53.7 per cent, for 0.10 cc. 52.5 per cent and for 0.05 cc. 49 per cent. Injecting the epinephrine at three different sites in divided doses did not change the response. In this case 0.15 cc. produced an average increase of 53.7 per cent. In subsequent experiments, because the larger doses were toxic and produced no greater response than the smaller ones, we have used 0.05 cc. of epinephrine as the test dose and have measured the response for two hours thereafter. The results for longer periods show the same relations as those described below but are less striking because the response diminishes rapidly after the first two hours. There is no evidence that the calorigenic response to epinephrine is shortened during increased thyroid activity or lengthened during decreased function.

That gelatin in the mixture accounts for no part of the metabolic response was shown by giving this without epinephrine. The basal metabolism during control measurements averaged 34.5 ± 0.6 cal. per sq. meter per hour and after the gelatin was 33.3 ± 0.5. The gelatin served only to slow the absorption of the epinephrine and prolong the effect so that this could be more easily and accurately measured.

In the first table we have shown the response to epinephrine when varying amounts of thyroid hormone (or thyroxin) are present in the body. It will be noted that the degree of increase in calorigenic response to epinephrine with in-

creasing thyroid activity varies as the height of the basal metabolism. This effect is clearly brought out by the straight line obtained when plotting the logarithm of the response against the basal metabolism.

TABLE 1

Effects of changes in thyroid activity on the calorigenic response to epinephrine (0.05 cc. suprarenalin in gelatin)

RAT NO.	BASAL METABOLISM*	AVERAGE INCREASE* DURING 2 HOURS AFTER EPINEPHRINE INJECTION	INCREASE	DOSE OF THYROXIN	BASAL METABOLISM*	AVERAGE INCREASE* DURING 2 HOURS AFTER EPINEPHRINE INJECTION	INCREASE
		Hypothyroid rats					
			per cent	*mgm.*			*per cent*
1	25.7	7.6	29.5				
2	25.8	12.8	49.5				
3	26.0	15.1	58.0				
4	26.8	12.7	47.4				
5	27.3	10.8	39.5				
6	28.5	10.3	36.2				
Average....	26.7	11.6	43.4				
		Normal rats			Normal rats one week after receiving thyroxin		
7	35.2	19.1	54.2	0.5	39.5	17.3	43.8
8	31.3	19.8	62.8		36.4	21.6	60.9
9	36.7	18.3	49.8		37.8	22.2	58.7
10	27.2	14.3	52.5		36.6	28.6	78.1
				Average... 4	37.9	22.4	60.3
11	32.3	19.4	60.0		39.9	26.2	65.7
12	32.2	14.6	45.3		44.5	23.4	52.8
Average....	32.4	17.6	54.1	Average... 8	42.2	24.8	59.3
					51.5	42.7	82.9
					43.1	55.7	129.0
					52.7	37.3	72.8
				Average...	49.1	45.2	94.9

* Calories per square meter per hour.

The above relationship between the basal metabolism and the epinephrine response is not shown when the change in metabolism is due to non-thyroid factors which we have tried. Table 2 shows the effect of epinephrine in animals in which the metabolic rate is raised by dinitrophenol (2 mgm. per 100 grams). This drug quickly elevates the metabolism and its effect continues over a period of several hours. In estimating the response to epinephrine superimposed upon

the dinitrophenol effect, suitable corrections have been made for the progressively diminishing calorigenic effect of the latter drug. If this had not been done, the contrast between thyroxin and dinitrophenol would have been even more striking. Nevertheless, in only one of seven observations was the per cent increase in metabolic response to epinephrine greater in a dinitrophenol-treated animal than in one not so treated. In four cases even the absolute increase in

TABLE 2

Calorigenic effect of epinephrine after dinitrophenol

RAT NO.	BEFORE DINITROPHENOL		AFTER DINITROPHENOL	
	Increase*	Increase	Increase*	Increase
	cal.	*per cent*	*cal.*	*per cent*
20	16.3	53.2	15.3	39.5
21	14.0	40.0	17.2	38.2
22	13.8	37.6	15.7	32.2
23	17.2	46.2	28.6	63.0
24	22.1	59.5	25.7	57.5
25	22.6	69.8	22.2	49.4
26	19.9	58.0	13.2	27.2
Average........	18.0	52.0	19.7	43.9

* Average increase in calories per square meter per hour during the two hour interval following the injection of epinephrine. (The dinitrophenol raised the basal metabolism an average of 34 per cent. The epinephrine effect was superimposed upon this.)

TABLE 3

Calorigenic effect of epinephrine after fasting for 3 days

RAT NO.	BASAL METABOLISM*	INCREASE AFTER EPINEPHRINE*	INCREASE
			per cent
A	25.7	15.8	61.5
B	25.2	14.6	57.8
C	30.7	14.9	48.6
D	28.6	11.9	41.6
E	23.8	6.7	28.2
F	23.6	15.7	66.5
Average.............	26.3	13.3	50.7

* Calories per square meter per hour.

metabolism was smaller. Table 3 shows the results on fasted animals. The basal metabolism was about the same as in the hypothyroid rats but the stimulation due to epinephrine was on the whole greater both in per cent and in calories. In only one rat of the six studied was the calorigenic response to epinephrine less than the average for hypothyroid rats. These results suggest that the type of response shown in table 1 may be specific for variations in thyroid activity.

The importance of the thyroid gland in the calorigenic response to cold is shown in table 4 in which the maximal catabolic response to cold during an exposure lasting for about three hours is shown. These results would probably have been more striking if larger doses of thyroxin had been used. This was not tried because the lowest temperature which could be obtained in the water bath used did not give maximal metabolic stimulation when the basal metabolism was very high. It will be noted that in eight of nine rats given thyroxin the increase in metabolic rate produced by cold was greater after the injection than before.

TABLE 4

Maximal catabolic response to cold with changes in thyroid activity

RAT NO.	BASAL METABOLISM[*]	INCREASE[*] DURING EXPOSURE TO COLD	BASAL METABOLISM[*]	INCREASE[*] DURING EXPOSURE TO COLD
	Thyroidectomized rats			
		mel.		*mel.*
30	23.9	46.6		
31	25.2	43.2		
32	26.8	46.1		
Average...............	25.3	45.3		
	Normal rats		Normal rats one or two weeks after receiving 0.3 mgm. thyroxin	
33	32.2	51.2	33.7	52.8
34	34.3	57.0	34.4	60.7
35	32.0	61.2	33.8	69.3
36	32.8	50.2	34.4	53.0
37	31.0	65.3	32.0	74.5
38	36.5	48.3	37.4	58.0
39	28.5	64.7	28.1	61.0
40	35.2	51.2	33.9	58.2
41	34.1	55.7	34.4	59.8
Average...............	33.0	56.1	33.6	60.8

* Calories per square meter per hour.

The thyroidectomized rats all showed smaller responses than any of the normal controls.

This metabolic response to cold is due in part to shivering and in part to the release of adrenine. Other factors may also be involved. It would be interesting to know the relative importance of the adrenal medulla but we were unable to devise an accurate method for determining this, since after ablation of the adrenal medulla, more energy may be released by shivering and made available for maintaining body temperature (see Cannon, Newton, Bright, Menkin and Moore, 1929). We did find, however, that in spite of this, the response to cold was slightly reduced by demedullation. The average maximal increase in metabolism in response to cold was 49.5 cal. per sq. m. per hour in the operated rats as compared with the normal response of 56.1 cal. To make sure that this was

due to a lack of adrenine and not a deficiency of cortical principle, we have given cortical extract to the operated rats. This produced no improvement in the response to cold. Another deficiency in the rats lacking secretion of adrenine is not brought out by these observations. It is the inability to maintain this subnormal rate of heat production for even a few hours. After three hours in the cold, the body temperature of the normal rats was usually 33 or 34° C. but in the demedullated rats it had fallen below 30°. This perhaps indicates the

TABLE 5

Average calorigenic effect* of 0.05 cc. epinephrine following three weeks' exposure to cold

TIME AFTER LEAVING REFRIGERATOR	BASAL METABOLISM	INCREASE AFTER EPINEPHRINE (FIRST 2 HRS.)
24 hours	39.7	31.1 ±1.1
1 week	38.0	32.2
2 weeks	36.6	32.5
3 weeks	33.1	33.4
4 weeks	34.1	27.9
5 weeks	31.5	27.2 ±1.9
6 weeks	34.8	32.2
7 weeks	33.4	31.0
9 weeks	32.9	23.9 ±1.2
10 weeks	32.3	26.4
11 weeks	33.1	27.2

* Obtained from observations on 8 rats.

TABLE 6

Basal metabolism and response to epinephrine when sodium iodide* is given after exposure to cold. (Average results from observations on 6 rats)

TIME AFTER LEAVING REFRIGERATOR	BASAL METABOLISM	INCREASE AFTER EPINEPHRINE
24 hours	37.2	38.2
1 week	37.6	37.7
2 weeks	33.1	31.5
3 weeks	34.3	26.2
4 weeks	34.1	32.7
5 weeks	35.8	32.8
6 weeks	34.7	30.4

* Seventy-five-hundredths milligram per cubic centimeter in drinking water.

importance of the defatiguing effect of adrenine during shivering (see Luco, 1939).

When a rat is exposed to cold for three weeks or more, the thyroid is stimulated and as one would expect the response to epinephrine is potentiated. The results are shown in table 5. As thyroid activity diminishes with the return of the rat to normal environmental temperature, the calorigenic effect of epinephrine is also reduced. The time required for these changes is shortened if NaI is given (table 6). These results when compared with those in table 1 suggest that the thyroid principle is alone responsible for the elevation in metabolism and the sensitivity to epinephrine.

DISCUSSION. The results given in table 1 show that the calorigenic response to epinephrine increases as the amount of thyroid principle present in the body increases. The amount of thyroid hormone present can be estimated from the observations on basal metabolism if we accept the work of Gaddum and Hetherington (1931), or Meyer and Wertz (1939). These authors showed that the increase in basal metabolism produced by thyroid preparations was proportional to the logarithm of the amount of thyroid principle given. Biilman (1937) showed that the blood organic iodine bears this same relation to the basal metabolism. If one plots the amount of thyroid hormone estimated to be present in the body against the response to epinephrine, one obtains a hyperbolic curve. It is therefore possible that a reversible chemical reaction between epinephrine and thyroid hormone or adsorption may be involved in this change in calorigenic response to epinephrine with changing thyroid function. The data on maximal metabolic response during short exposures to cold shown in table 4 are not sufficiently complete but they may also be represented by a hyperbolic curve.

CONCLUSIONS

1. The calorigenic response to epinephrine is potentiated by thyroid hormone (see table 1).

2. Every rise or fall in basal metabolism does not bring about a similar increase or decrease in the response to epinephrine (see tables 2 and 3). Therefore the sensitizing effect may be specific for thyroid hormone.

3. The maximal catabolic response to cold is potentiated by thyroid hormone (see table 4).

4. The secretion of the adrenal medulla may account for 10 per cent of the increased metabolism produced by cold. It is also important in maintaining an elevation in metabolism.

5. After a prolonged sojourn in a refrigerator, the calorigenic effect of epinephrine in rats is about as much as would be expected from the elevation in basal metabolism (compare tables 5 and 1).

6. Following prolonged exposure to cold, the basal metabolism and the calorigenic effect of epinephrine become normal more quickly if extra NaI is given (see table 6).

REFERENCES

(1) BENEDICT, F. G. AND G. MACLEOD. J. Nutrition 1: 367, 1929.
(2) BIILMAN, G. Skand. Arch. f. Physiol. 77: Suppl. 74, 1937.
(3) CANNON, W. B., H. F. NEWTON, E. M. BRIGHT, V. MENKIN AND R. M. MOORE. This Journal 89: 84, 1929.
(4) GADDUM, J. H. AND M. HETHERINGTON. Quart. J. Pharm. and Pharmacol. 4: 183, 1931.
(5) MEYER, A. E. AND A. WERTZ. Endocrinology 24: 683, 1939.
(6) RING, G. C. This Journal 122: 435, 1938.
(7) RING, G. C. This Journal 125: 244, 1939.
(8) RING, G. C. This Journal 131: 357, 1940.
(9) RING, G. C. This Journal 134: 631, 1941.
(10) SWIFT, R. W. AND R. M. FORBES. J. Nutrition 18: 307, 1939.

PREVENTION OF EXPERIMENTAL SHOCK FOLLOWING VENOUS OCCLUSION IN THE DOG BY THE APPLICATION OF A RIGID CAST[1]

L. N. KATZ, I. H. SHLESER, R. ASHER AND S. PERLOW

From the Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

Previous work from this department (1, 2) has shown that venous occlusion of the limb in the dog led to a shock-like state and death in $3\frac{1}{2}$ to 21 hours. This was associated with a loss of intravascular fluid into the leg of more than 4 per cent of the body weight, which was attributed to the loss of plasma fluid caused by the elevation of the hydrostatic pressure in the small peripheral vessels. Later alterations in capillary permeability lowered the osmotic pressure of the blood and permitted proteins to escape and still later even the formed elements. The possibility, however, that the shock-like state and death were accelerated by the absorption of noxious materials from this leg through newly created venous collaterals has not been excluded. The establishment of a return venous flow is indicated by the resorption of the swelling in the leg in animals surviving venous occlusion of the limb after priming with desoxycorticosterone acetate (2). If such a humoral factor plays a significant rôle in the syndrome following venous occlusion in the limb, some evidence of shock should be manifest even if the local loss of plasma fluid is prevented. This was the purpose of the present study in which the local loss of plasma fluid was restrained by encasing the limb in a plaster cast, which prevented the swelling ordinarily following venous occlusion.

METHOD. The procedure used in occluding the venous drainage of the dog's hind leg has been described previously (1, 2). Similar observations were made in the present study except that it was not possible to obtain blood pressure readings during the 36 hours that the cast was in place since both lower limbs were enclosed. Immediately after the operation was completed the cast was applied to both lower limbs and to the groin and lower abdomen.[2] The tail was placed along with the unoperated leg and enclosed in the cast. No opening was made for urination and defecation because local edema, protrusion and constriction of the extruded tissue occurred when an opening was left; the animal could perform these excretory functions inside the cast. In the first 7 dogs the extremities were covered with a stockingette and then wrapped with plaster bandage. Since gangrene of the toes of the occluded limb occurred in 3 of the animals, a preliminary wrapping of raw cotton was used in the last 6 dogs. This cotton provided a yielding medium permitting some expansion without severe compression of the

[2] This extensive casting was used because in a preliminary experiment it was shown that fluid escaped above the cast into the groin, belly wall and down the other leg when the cast was applied only to the leg with the occluded vein.

smaller distal vessels and was successful in preventing the gangrene. After 36 hours the cast was removed, the previous observations on the clinical state of the animal and on the hematrocrit were continued and determinations were made of the blood pressure and of the rate of limb enlargement.

RESULTS. Of the 13 dogs used, 11 survived, and only one died in shock 12 hours after operation.[3] One other animal died of bronchopneumonia in 32 hours. This low mortality is in sharp contrast to our control series (1, 2) in which 13 out of 15 dogs died in shock within $3\frac{1}{2}$ to 21 hours. The animals with casts generally ate and drank well, and were not apathetic. In fact several dogs, including the one which died in shock, were unusually active in attempting to remove the cast.

No hemoconcentration occurred in these dogs, not even in the one succumbing in shock. By the second day all of the 12 dogs showed varying degrees of hemo-
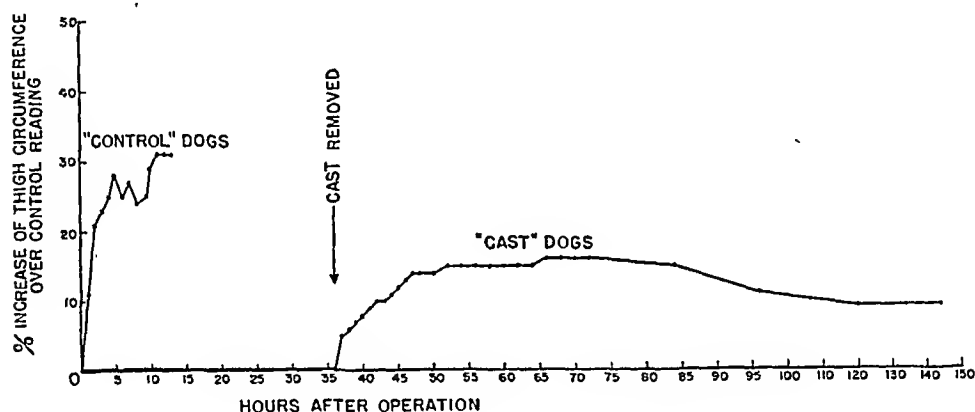
Fig. 1. A comparison of the average increase in size of leg with venous occlusion in the control series (without casts) previously reported (2) with that in the present series in which a cast was applied for 36 hours. Zero time is the time at which the operation ended. Discussed in text.

dilution which continued for several days. The arterial blood pressure showed no significant deviation from normal after the cast was removed over the several days during which observations were made. No limb enlargement was found, the circumference at mid-thigh and at mid-calf of the limb with occluded veins and the control limb were the same as before operation. However, over the next few hours some enlargement of the operated limb was found (fig. 1) but this was in no way comparable to the previous control series (2). This enlargement had no demonstrable effect on the animal's condition, nor did it lead to a hemoconcentration or a drop in blood pressure, even though in four dogs the leg reached a large size, viz., 125 per cent, 126 per cent, 130 per cent and 143 per cent of the control, respectively.

DISCUSSION. The absence of shock in all but one of these animals with venous occlusion when a cast is used to prevent the local accumulation of fluid in the leg

[3] This animal had no leg enlargement.

is in sharp contrast to the development of a shock-like state and death following the same procedure in 13 out of 15 dogs when the cast is omitted. This suggests that the local accumulation of fluid is the primary, if not the sole, precipitating factor in causing this form of shock and in making it irreversible. These findings also rule out the possibility that neurogenic or humoral factors play more than a minor rôle in producing peripheral vascular collapse and in making shock irreversible. The application of the cast should have no significant effect on neurogenic impulses nor on the absorption of noxious substances from the leg into the blood stream. Hence it follows that the entire sequence is initiated and perpetuated by the local loss of plasma fluid.

These experiments, therefore, support our previous deduction (1, 2). With the disturbances set up by the local accumulation of plasma fluid, a series of interrelated changes is established, the details of which are not known, which perpetuate, aggravate and ultimately make the state of shock irreversible.

There can be no doubt that the ether anesthesia used in this operation may have contributed to the picture and this may help to explain the death of one of the "cast dogs." However, it requires more definite evidence than is at present available to ascribe this animal's death to a toxic factor.

The effectiveness of this method in preventing local loss of fluid was demonstrated clearly by the absence of leg enlargement when the cast was removed. However, leg enlargement appeared soon after removal of the cast. While in several of these animals the leg became large, in none was the rate of fluid loss as rapid as in the controls (fig. 1). Apparently, during the first 36 hours of casting sufficient venous collaterals had developed to improve the local circulation so that the hydrostatic pressure rise in the capillaries was lessened, and less local anoxia and impairment of capillary permeability resulted, thus decreasing the rate of fluid loss. The absence of shock after removal of the cast emphasizes that local accumulation of fluid determines this state not so much by the *amount* of plasma fluid lost but rather by the *rate* at which it is lost. Apparently, compensatory factors can keep pace when the rate is slow, but above a *critical rate* they fail to do so and shock is initiated, perpetuated and becomes irreversible.

Certain practical clinical implications are indicated by this study. They suggest the utility of applying a plaster cast to severely injured limbs in which plasma fluid loss may conceivably contribute to the development of shock. This is a simple procedure to carry out on man even away from the hospital, and should be seriously considered.

It appears to us that some of the benefits reported by Trueta (3, 4) in cases in which crush injuries of the limb were cleansed, the debris removed and the leg immediately casted over the open wounds may be due to prevention of local fluid accumulation. In such treated cases shock was rare, one instance in 1073 cases.

Recently, the idea of eliminating the local fluid accumulation has been applied to crush injuries during air raids. This was accomplished by using a large blood pressure cuff connected to a pump intermittently raising its pressure in order to "milk" the edema from the injured limb (5). It was found that this procedure

prevented the occurrence of shock, whereas routine anti-shock measures in these crush cases did not prevent a fatal outcome. However, there is the danger of releasing emboli from intravenous thrombi which might be present as a result of the crush. It appears to us that fluid accumulation should be *prevented* by the immediate use of a cast rather than "milking" by intermittent external pressure.

Duncan and Blalock (6) have reproduced severe crushing injuries in dogs and found that the use of a rubber boot, inflated to 40 mm. Hg, over the crushed limb decreased mortality from 95 per cent to 33 per cent. This decline in mortality was ascribed to the prevention of local fluid accumulation in the limb. Our results suggest that the same benefit might have been obtained by a plaster cast which is simpler and more readily applied. We see no need of raising the pressure as high as 40 mm. Hg. In fact by so doing circulatory embarrassment occurs which might be detrimental because it would further increase the local anoxia.

The applicability of this casting procedure needs further exploration clinically both in civilian and military crush injuries of limbs.

SUMMARY

1. In a series of 13 dogs the application of a plaster cast for 36 hours to the lower extremities led to the survival of 11 animals following venous occlusion of the limb. Only one dog died in shock. This contrasts with the development of shock in 13 out of 15 dogs following this operation when no cast is applied, death occurring in $3\frac{1}{2}$ to 21 hours.

2. These results indicate that the cast by preventing the local accumulation of plasma fluid avoided the shock syndrome.

3. The local fluid accumulation which occurred following the removal of the cast developed at a slower rate than in the control series. The absence of untoward results in the period following removal of the cast suggests that for the shock syndrome to become established the loss of plasma fluids must occur at a rapid rate, a rate faster than compensating mechanisms can cope with.

4. This casting procedure appears to be applicable clinically for use in both civilian and military crush injuries.

REFERENCES

(1) PERLOW, S., S. T. KILLIAN, L. N. KATZ AND R. ASHER. This Journal **134**: 755, 1941.
(2) KATZ, L. N., S. T. KILLIAN, R. ASHER AND S. PERLOW. This Journal **137**: 79, 1942.
(3) TRUETA, J. Lancet **1**: 1452, 1939.
(4) TRUETA, J. British Med. J., 616, 1942.
(5) PATEY, D. H. AND J. D. ROBERTSON. Lancet **1**: 780, 1941.
(6) DUNCAN, G. W. AND A. BLALOCK. Ann. Surg. **115**: 684, 1942.

SOME EFFECTS OF SULFATHIAZOLE AND SULFADIAZINE ON MAN AT REST AND DURING EXERCISE

F. J. W. ROUGHTON, R. C. DARLING, W. H. FORBES, S. M. HORVATH, SID ROBINSON AND J. H. TALBOTT

From the Fatigue Laboratory, Morgan Hall, Harvard University, Boston, Massachusetts

Sulfanilamide, in the dosage customarily given to ambulant patients, has been shown by Roughton et al. (1) to reduce the capacity both for exacting mental work and heavy manual labor. The deleterious effect in the latter instance was shown to be in part due to interference with the normal rate of CO_2 removal, owing to inhibition of the carbonic anhydrase of the red cells (2). It therefore became of interest to test, in a similar manner, the effect of ambulatory doses of sulfathiazole and sulfadiazine, since these two drugs are *1*, without inhibitory effect on carbonic anhydrase in vitro, and *2*, are regarded as superior to some of the earlier drugs of the sulfanilamide series both in therapeutic action and freedom from toxic consequences. They are, indeed, in widespread use amongst military and industrial personnel, particularly for the treatment of gonorrhea, and a study of their exact effect on the working capacity of ambulant patients is therefore of obvious importance at the present time.

PROCEDURES. Except in a few details the experimental schedule was identical with that used for studying sulfanilamide. The subjects were six normal male members of the laboratory staff, whose ages were between 23 and 40. Three of them received sulfathiazole and three sulfadiazine. Except in the case of *JT* who took the drug for only two days, all received it for three days, 3.0 grams on the first day, 2.0 grams on the second and 4.0 grams on the third, the daily amount being divided into four or six equally spaced doses on each day. Complete tests according to the outline below were performed on each subject before starting the drug, after 24 hours and after 72 hours of the drug, and in some cases several days after stopping the drugs. As a test of mental ability and coordination, the Johnson Code Test was carried out daily in triplicate for three days preceding the drug, during its administration and for three days afterward. Four additional subjects, not receiving the drugs, were tested daily by the code test as controls during the same period. The Johnson Code Test was the only mental test used, as it alone of the three tried in the sulfanilamide work gave positive results.

The following is an outline of the procedures for the main tests performed on at least three days on each subject.

A. *Under resting conditions.* 1. Basal ventilation, oxygen consumption and CO_2 output were obtained from a ten minute collection of expired air in a Tissot Gasometer. Pulse and respiratory rates were recorded.

2. Arterial blood samples were taken under oil and mixed with heparin. O_2 and CO_2 content were determined by the Van Slyke technique on a portion of the

blood. The remainder of the blood was equilibrated in a tonometer at 37° ($pCO_2 = 40$, $pO_2 = 180$) and then analyzed for CO_2 and O_2. From these analyses the alkali reserve (T_{40}), pCO_2, HbO_2 saturation and pH_s of the arterial blood were calculated (Dill et al., 3).

3. Alveolar gas samples were obtained simultaneously with the arterial punctures.

B. *Bicycle exercise.* (A bicycle ergometer was used with a mechanical brake. The brake tension was maintained at five pounds. This exercise required a metabolism five to six times the basal figure.)   1. Ventilation, oxygen consumption and CO_2 output were measured from a collection of expired air between the 9th and 14th minutes of the exercise.

2. Pulse rate was noted every five minutes throughout.

3. Arterial blood and alveolar gas samples were collected after 15 minutes and analyses carried out as with the basal samples. Bloods were analyzed also for lactate and sugar.

4. Exercise was stopped and rectal temperature was determined immediately.

C. *Exhausting treadmill exercise.* (The treadmill grade was 8.6 per cent, the speed was 5.8 or 7.0 m.p.h. depending on the ability of the subject. The work was sufficient to exhaust the subjects within five minutes.)   This exercise was begun only after at least 30 minutes' rest following the previous exercise.   1. Ventilation was measured for each half-minute, and expired air samples were taken for analysis for each whole minute after the first.

2. Pulse rate was recorded throughout by a Guillemin cardiotachometer. From each record the maximum rate was obtained.

3. As soon as exhaustion forced the subject to stop he sat down and the pulse was counted for three one-minute periods: $\frac{1}{2}$ to $1\frac{1}{2}$ minutes, 2 to 3 minutes and 5 to 6 minutes after stopping. It has been found in this laboratory (4) that these three values give the main characteristics of the pulse recovery curve.

4. Capillary blood from the finger was then obtained for lactate and sugar determination.

RESULTS. The quantitative results as well as the subjective reactions to these drugs were remarkable chiefly by the extremely small changes from the normal state. In contrast to the feelings after sulfanilamide no subject complained of any severely disturbing symptoms; all carried on their usual activities. Three subjects (two on sulfathiazole and one on sulfadiazine) noted an increase in the number of daily bowel movements together with some uncomfortable flatus. In no case was there true diarrhea, nausea, vomiting or anorexia. Several subjects said that they had slight malaise, a vague feeling of being below par. However, SR, who had the highest blood level of the drug, noted no unusual subjective sensations.

Table 1 presents a part of the results of metabolic studies and blood analyses. It is presented in a form similar to the previous report on sulfanilamide so as to facilitate comparison. It includes many negative results together with all those showing any changes. Of the measurements and observations not presented in tabular form, none showed any suggestion of significant change.

TABLE 1

Summary of data on subjects before, during and after administration of sulfathiazole and sulfadiazine

SUBJECT	DRUG	NO. OF DAYS	BLOOD LEVEL (mgm.%)	RESTING — Ventilation S.T.P. (l./min.)	RESTING — CO2 min. (ml.)	RESTING — O2 min. (ml.)	RESTING Arterial pCO2 (mm.Hg)	RESTING Arterial T10 (vol.%)	RESTING Arterial pHs	BICYCLE Pulse rate per min.	BICYCLE Ventilation S.T.P. (l./min.)	BICYCLE CO2 min. (l.)	BICYCLE O2 min. (l.)	BICYCLE Arterial pCO2 (mm.Hg)	BICYCLE Arterial T10 (vol.%)	BICYCLE Arterial pHs	BICYCLE Lactate (mgm.%)	TREADMILL Duration (min. and sec.)	Minute before last CO2 (l.)	Minute before last O2 (l.)	Minute before last R.Q.	Last minute CO2 (l.)	Last minute O2 (l.)	Last minute R.Q.	Lactate 6 min. after run (mgm.%)
J. T.	Pre-drug		0	5.07	192	244	39.8	49.5	7.43	129	28.5	1.20	1.30	38.3	45.5	7.40	32	3'30"	3.35	2.90	1.16	3.48	2.85	1.22	99
	Sulfadiazine	1	2.4	5.85	183	246	38.4	49.7	7.44	132	35.5	1.42	1.50	35.2	41.2	7.38	44	3'30"	3.31	2.92	1.13	3.48	2.94	1.18	90
	Sulfadiazine	2	3.7	6.20	194	253	42.3	47.6	7.38	138	31.7	1.25	1.41	38.0	44.2	7.39	30	4'	3.40	3.00	1.13	3.71	3.17	1.16	70
	Recovery	0	0	5.59	191	241				121	29.6	1.31	1.41				18								
S. R.	Pre-drug		0	5.91	194	238	39.8	48.3	7.41	140	44.9	1.70	1.73	31.0	37.5	7.35	73	5'	3.50	3.02	1.16	3.62	3.11	1.10	134
	Sulfadiazine	1	4.4	6.56	199	242	37.3	48.0	7.43	144	41.0	1.49	1.54	28.3 (?)	42.1	7.45 (?)	47	5'	3.54	3.06	1.16	3.02	3.19	1.10	127
	Sulfadiazine	3	5.8	0.31	192	252	36.2	47.8	7.44	118	28.0	1.08	1.24	33.5	42.0	7.41	34	5'	3.46	3.03	1.14	3.46	3.37 (?)	1.03	112
	Recovery	0	0	5.83	201	231				128	31.3	1.30	1.41				39								
F. C.	Pre-drug		0	0.81	252	293	41.2	47.0	7.39	114	31.1	1.38	1.55	38.0	45.5	7.41	24	3'31"	4.59	4.25	1.08	4.84	4.34	1.12	103
	Sulfadiazine	1	2.6	0.72	247	297	41.8	47.7	7.39	110	30.2	1.31	1.55	35.5 (?)	48.7	7.45 (?)	21	4'	4.63	4.38	1.06	4.93	4.53	1.09	100
	Sulfadiazine	3	4.5	0.61	247	291	42.4	46.6	7.38	100	25.6	1.11	1.32	40.9	46.7	7.39	14	4'8"	4.51	4.27	1.05	4.80	4.45	1.08	92
	Recovery	8	0	5.85	229	279				100	28.2	1.22	1.39				16								
R. D.	Pre-drug	0	0	5.88	235	294	35.0	49.2	7.45	136	40.7	1.58	1.73	36.9	44.0	7.39	30	2'30"	3.76	3.47	1.09	4.32	3.70	1.17	98
	Sulfathiazole	1	1.6	6.34	244	307	38.6	50.1	7.43	124	36.8	1.41	1.58	35.3	45.6	7.42	22	2'23"	3.75	3.73	1.00	4.29	3.50	1.22	100
	Sulfathiazole	3	2.1	5.94	225	280	38.7	48.6	7.41	120	34.3	1.35	1.56	36.0	45.5	7.41	23	2'35"	3.79	3.55	1.03	4.45	3.79	1.14	95
	Recovery	8	0							115	30.3	1.43	1.54				22								
G. T.	Pre-drug	0	0	5.28	189	230	44.8	49.8	7.39	116	33.1	1.57	1.69	37.3	44.6	7.41	38	5'	3.78	3.16	1.19	4.06	3.47	1.17	137
	Sulfathiazole	1	1.5	5.87	200	248	44.7	50.2	7.40	126	35.3	1.57	1.69		43.6		34	3'	3.19	3.19	1.07	3.47	3.04	1.18	99
	Sulfathiazole	3	3.3	4.80	214	259	43.3	50.7	7.41	140	30.6	1.37	1.46	38.1	47.8	7.42	24	4'3"	3.78	3.05	1.24	3.82	3.12	1.23	118
	Recovery	8	0	5.79	201	242																			
W. F.	Pre-drug	0	0	4.47	187	246	40.0	48.7	7.42	129	25.4	1.25	1.41	42.5	44.0	7.35	25	3'20"	4.09	3.23	0.99	4.09	3.47	1.18	118
	Sulfathiazole	1	1.7	5.22 (?)	194	271 (?)	40.1	48.4	7.41	128	26.4	1.21	1.40	38.4	45.5	7.40	23	2'34"	3.44	3.44	0.99	4.15	3.45	1.20	102
	Sulfathiazole	3	1.8	4.78	199	250	40.1	47.9	7.40	125	23.0	1.10	1.24	41.1	44.8	7.37	21	3'3"	3.40	3.40	0.97	4.07	3.35	1.21	114
	Recovery	0	0	4.57	180	238				110	23.4	1.21	1.34				14								

The blood levels of sulfathiazole and sulfadiazine ranged from 1.1 to 5.8 mgm. per cent, being higher as expected on the third day and higher dosage of the drug. The individual variations in drug concentrations on the same intake is in line with the commonly observed variations under therapeutic usage. Estimations of sulfhemoglobin and methemoglobin on the same blood samples showed no more than 0.2 gram per 100 cc. in any instance.

The basal oxygen consumption showed minor changes which were unexpected and not observed following sulfanilamide. There was an increase during drug

TABLE 2

Time in seconds* to complete Johnson Code Test

NAME	BEFORE	DURING	AFTER
A. Sulfadiazine			
S. R.	147	148	142
F. C.	106	105	106
Average..........	127	127	124
B. Sulfathiazole			
R. D.	93	91	92
E. T.	122	116	115
W. F.	102	108	110
Average..........	106	105	106
C. Controls			
S. M. H.	102	94	88
W. H.	164	157	157
J. R.	83	˯82	75
R. J.	112	96	94
Average..........	115	107	104

* Increased time indicates poorer performance.

administration in three of the six cases with a return to or below the previous level following cessation of the drug. In two other cases the rate of oxygen consumption following the drug was lower than the figures during either the first control or drug period. The maximum change in any case was about 8 per cent which is not a figure of great practical importance. We cannot state its cause or theoretical importance at present.

The analyses of the resting arterial bloods showed no regular changes. The maintenance of a constant alkali reserve (T_{40}) is in clear contrast to the effect of sulfanilamide. Alveolar pressures of CO_2 (not on table) agreed fairly well in all instances with the calculated pCO_2 of the arterial blood.

The changes noted under the bicycle exercise may all be logically attributed to a training effect (only one of the subjects FC was accustomed to bicycling) or to unavoidable slight variations in the brake tension. The effects of training are seen in a drop in the ventilation, the blood lactate and the oxygen consumption. This change in ventilation is in marked contrast to that following sulfanilamide which was an average increase of nearly 30 per cent.

The arterial blood during exercise shows again no changes attributable to the drug. Here one can again observe the effect of training in some subjects in that in the early experiments with higher blood lactate values the alkali reserve (T_{40}) was lower than in later tests. This is the reverse of the effect of sulfanilamide.

The alveolar CO_2 values in exercise (not in the table) were not as satisfactory technically as those at rest. Regardless of these difficulties one can still conclude that there were no differences between the drug and control periods.

The results from the exhausting runs show a remarkable constancy in gaseous exchange during the last two minutes, no trace of the failure in CO_2 removal or the drop of R.Q. observed following sulfanilamide. Likewise no feeling of suffocation was experienced as after sulfanilamide. There were unavoidable and expected slight variations in the time the subjects could run before exhaustion. In only one subject (GT) was there a significant decrease which in this case was probably due to an upper respiratory infection beginning after the control run.

The results of the Johnson Code Tests are presented in table 2. All changes recorded are small and probably not significant. In no case was there any obvious deterioration during the drug period. On the average the control (no drug) subjects showed a somewhat greater improvement in performance during the nine day test period than those taking the drugs. Whether this signifies a masking of improvement in the latter group by the drug can only remain an academic question in view of the small changes involved.

DISCUSSION. Under the conditions of these tests it appears that sulfathiazole and sulfadiazine are remarkably benign drugs in their effect on physical and mental performance. This is especially significant in view of the fact that the daily dosage on the third day was double that recommended for ambulant patients. It is obvious that these tests tell us nothing about the possible cumulative toxic effects from more prolonged administration. With ambulant patients on short term doses there appears to be no reason why their normal activities should be curtailed.

The difference between the effects of sulfanilamide on one hand and sulfathiazole and sulfadiazine on the other may be explained to a large extent by the fact that the former poisons the carbonic anhydrase of the body while the latter does not affect it. Although the mechanisms are not clear, it is possible to explain the drop in alkali reserve and the difficulties in CO_2 removal under sulfanilamide by this fact. The disturbance in mental capacity is not so directly or clearly related.

CONCLUSIONS

1. Sulfathiazole and sulfadiazine, in doses recommended for ambulant therapy and in double these doses for one day, caused only negligible symptoms and no limitation in the normal activity of six laboratory workers.

2. No disturbances in acid-base balance were observed either from the study of arterial blood or the metabolism at rest or in work.

3. The ability to perform moderate and exhausting work was not impaired by the drug, i.e., in CO_2 transport and removal.

4. Mental ability and coördination as tested by the Johnson Code Test were essentially unchanged by the drugs.

5. These findings contrasted markedly with the results of sulfanilamide which caused disturbances in all the categories above, especially in the exhausting work.

6. Sulfathiazole and sulfadiazine may be safely administered up to 4.0 grams per day (at least as far as immediate effect is concerned) to those doing heavy manual labor or exacting mental work.

REFERENCES

(1) ROUGHTON, F. J. W., D. B. DILL, R. C. DARLING, A. GRAYBIEL, C. A. KNEHR AND J. H. TALBOTT.   This Journal 135: 77, 1941.
(2) MANN, T. AND D. KEILIN.   Nature 146: 164, 1940.
(3) DILL, D. B., A. GRAYBIEL, A. HURTADO AND A. C. TAQUINI.   Ztschr. f. Altersforsch. 2: 20, 1940.
(4) JOHNSON, R. E., L. BROUHA AND R. C. DARLING.   Revue Canadienne de Biol. (in press).

ON THE CARDIOVASCULAR ACTION OF BILE SALT WITH REGARD TO INHIBITION OF CHOLINESTERASE

M. SCHACHTER AND S. DWORKIN

With the technical assistance of ESTHER DWORKIN

From the Department of Physiology, McGill University, Montreal, Canada

The systemic effects of bile (whole bile, bile acids, bile salts) have been studied by many workers. Within the circulation the substance is universally regarded as toxic, inducing severe cardiovascular and nervous depression (cf. Sollmann, 1942). The mechanism of the action is, however, still in doubt. Bradycardia, a prominent feature of biliary obstruction, was ascribed by Löwit (1882) to an effect upon vagal centers. Weintraud (1894) was able to abolish the slow pulse of clinical icterus by means of atropine. On the other hand, Wakim, Essex and Mann (1939) found that even the isolated perfused heart was usually depressed by bile salt, although occasionally they observed initial increases in rate and amplitude. Horrall and Carlson (1928) reported that the slowing of the heart which occurred immediately after the intravenous administration of bile salt was modified by vagotomy, while the whole inhibitory effect was prevented by atropine. The even more important hypotensive action of bile salt (Meltzer and Salant, 1905) was shown by Still (1929) to be largely independent of atropine or vagotomy. Baltaceano and Vasiliu (1934) attributed the fall of blood pressure to "increased sensitivity of carotid sinus and aortic nerves."

The question of the mechanism of action of the bile salts upon the body at large has gained renewed interest from recent work which suggests a relationship between bile-salt action and the acetylcholine-esterase system. Antopol *et al.* (Antopol, Tuchman and Schifrin, 1937; Antopol, Schifrin and Tuchman, 1938) reported low cholinesterase values in the blood serum of patients with liver and biliary disease. The characteristic circulatory depression might therefore be ascribed to a greater than normal concentration of acetylcholine in the circulation, a direct result of the failure of esterase. In a further study Sobotka and Antopol (1937) concluded that bile salt can inhibit the action of cholinesterase *in vitro*. More recently McArdle (1940) observed a greatly depressed cholinesterase activity in parenchymatous liver disease (hepatitis, cirrhosis, metastases) but not in simple obstructive jaundice. Since serum with low esterase values did not on dialysis lose the inhibitory substance, McArdle concluded that the deficiency of esterase in the conditions mentioned was not due to an "enzyme inhibitor."

The essential point of the hypothesis under consideration is whether bile salt does indeed induce cardiovascular depression by inhibiting the cholinesterase power of blood or other tissues. We have studied this problem in two types of experiments, one in the whole animal, the other on the isolated frog rectus abdominis muscle (an indicator of acetylcholine).

I. *Mechanism of systemic action of bile salt.* A series of observations was made on dogs and cats under nembutal anesthesia, in whom changes in blood pressure, heart rate and respiration were recorded. We noted first the effects on the functions mentioned of bile salt itself, then the effects of acetylcholine given after the bile salt. Secondly, we studied the effects of atropine, of vagotomy, and finally of destruction of the spinal cord upon the animals' response to bile salt. The dogs received, as a rule, 120 mgm., the cats 30 mgm. of bile salt per whole animal.

1. *The hypotensive action of bile salt.* The most consistent and striking action of bile salt, injected intravenously, was fall of the blood pressure.[1] The extent
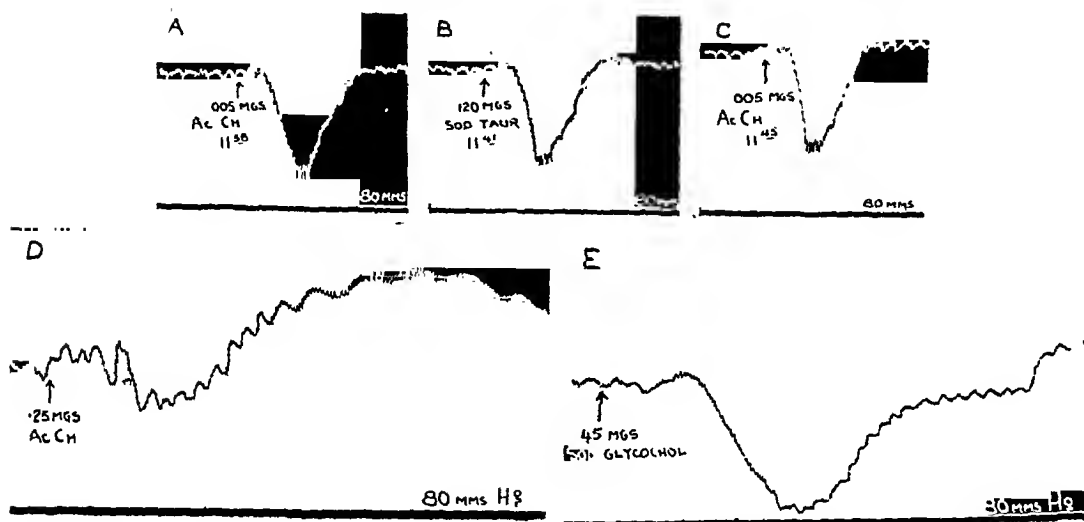
Fig. 1. A, B and C compare the hypotensive action of single doses of acetylcholine and sodium taurocholate. The three curves obtained in the same subject (dog, 15 kgm.) at four-minute intervals show that the effect of acetylcholine is not enhanced by the previous administration of bile salt.

D shows the well known "nicotine action" elicited by acetylcholine after previous administration of atropine (in a cat). The depressor is largely converted into a pressor effect.

E depicts the persistent depressor effect of bile salt after atropine injection (in a cat).

of the fall was proportional to the dose. It began within a few seconds and (depending on the amount injected) lasted up to three minutes after completion of the injection. There was evidence too of differences in susceptibility, the more sensitive cats showing a good depressor response to 3 or 4 mgm. The hypotensive action of bile salt resembled the fall of blood pressure produced by suitable doses of acetylcholine, the effective dose for the latter being, however,

[1] Rarely the bile salt caused, in both species, a definite pressor response, sometimes accompanied by increased rate and amplitude of the heart-beat. This phenomenon was noted many years ago by Landois (1863). In our experiments the pressor and augmentor effects were most apt to occur on the first of a series of injections, then to disappear and to give way to the more typical depressor response.

much smaller. Even the shape of the blood-pressure curve was frequently similar for the two substances (fig. 1, A, B, C). Table 1 shows the extent of the fall of blood pressure caused by different doses of the two substances (given separately).

2. *The influence of bile salt on heart action.* In only 8 out of 21 experiments was fall of blood pressure accompanied by slowing of the heart. When present, the reduction in rate was evident immediately after the injection and was usually followed by a marked acceleration. There were no disturbances of rhythm. The rate of the slowed heart varied from 40 to 70 per cent of the normal rate.

3. *The effects of atropine, vagotomy and destruction of the spinal cord on the cardiac and hypotensive action of bile salt.* Several experiments were carried out to ascertain whether the effects of the bile salt were mediated by central vagus or peripheral mechanism. The slowing of heart rate was regularly prevented by section of both vagus nerves or by atropine (1 to 2 mgm.), as shown in

TABLE 1

ANIMAL	MGM. BILE SALT INJECTED	FALL OF BLOOD PRESSURE	MGM. ACETYLCHOLINE INJECTED	FALL OF BLOOD PRESSURE
		mm. Hg		*mm. Hg*
Dog	120	55	0.005	55
Dog	180	40	0.07	45
Dog	120	40	0.25	90
Cat	30	90	0.008	80
Cat	30	95	0.002	50
Cat	30	50	0.06	95
Cat	4	25	0.002	85
Cat	30	55	0.003	80
Cat	30	110	0.008	45
Cat	30	80	0.25	90

figure 2, A, B. It was therefore primarily of central vagus mediation. With very large doses of bile salt, however, we found, in confirmation of previous results, that slowing of the heart persisted after vagotomy. This direct action upon heart muscle would appear to be really toxic.

The hypotensive action of bile salt still persisted after any or all of the above procedures. It remained virtually unimpaired after injection of atropine, and in all but three instances after double vagotomy. In these exceptional cases where section of the vagus nerves prevented most of the fall of blood pressure one might suppose stimulation of the sino-aortic mechanism by the bile salt (cf. Baltaceano and Vasiliu, *loc. cit.*).

The most illuminating evidence as to site of the hypotensive action came from experiments in which the spinal cord was pithed. Here, in spite of the fact that the initial blood pressure was already very low, we still were able to elicit a definite reduction of the pressure with the usual amounts of bile salt (see fig. 2, C, D, E, F).

One may thus conclude that, while the cardiac slowing after injection of bile salt is mediated by central vagus, the hypotensive action is essentially a peripheral affair.

4. *The possible prostigmine- (eserine-)like action of bile salt.* Preliminary "calibration" tests were made to establish definite responses of blood pressure to known amounts of acetylcholine and of bile salt. Then the bile salt was injected and followed at different time-intervals by acetylcholine. Whether elicited 1 minute or 35 minutes after the administration of bile salt, the response to acetylcholine was not significantly changed. This failure of potentiation by
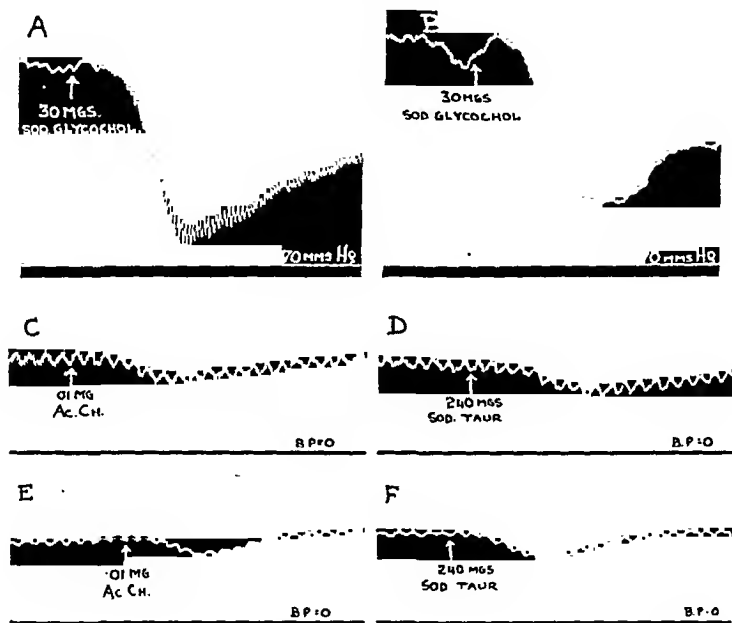
Fig. 2. A and B show the effect of vagotomy upon the cardiac slowing induced by injection of bile salt (in a cat). In A the vagi are intact, in B, divided. The depressor effect persists but the heart is not slowed.

C and D show that after high cervical section of the spinal cord (in a dog, 15 kgm.), despite the low level of the blood pressure, acetylcholine and sodium taurocholate still exert a depressor effect.

In E and F the depressor action of both substances is still evident in the same animal after spinal pithing.

bile salt was in marked contrast to the influence of prostigmine itself which, in the same subjects, gave the expected great exaggeration of the response to acetylcholine. Of further interest were our experiments with atropine. This drug readily abolished the enhancing action of prostigmine, and not merely prevented the depressor response to acetylcholine but even converted the effect of the latter to a pressor response (nicotine action) (fig. 1, D). The depressor action of bile salt was, however, little if at all modified by atropine. A typical result is shown in figure 1, E.

From the above experiments two conclusions can be drawn: 1, bile salt does not exert a potentiating (eserine-like) influence upon circulating acetylcholine,

and therefore the characteristic hypotensive action of bile should not be attributed to inhibition of cholinesterase; 2, the action of bile salt, though effectively similar to that of acetylcholine, is not exerted through the latter agency.

II. *Action of bile salt on the isolated frog rectus abdominis muscle.* In this series of tests we studied the action of bile salt upon contraction of frog rectus, then the potentiating action of bile salt upon acetylcholine, and finally compared the preserving ability of bile salt with that of prostigmine upon quantities of acetylcholine added to whole blood.

1. *Bile salt causes contraction of frog rectus abdominis.* It was first noted that bile salt itself, in sufficient quantities, can cause contraction of the excised muscle. Our average threshold figure for frog rectus was about 9 mgm. in 7 cc. of Ringer's solution. This concentration caused a just perceptible contraction, while larger quantities caused correspondingly greater effects.

The contraction of frog rectus by bile salt is, however, not due to intervention, or to potentiation of acetylcholine. The curves in the case of the two agents were not at all similar, that for bile salt being more gradual and taking an infinitely longer time to return to the base line. This latter feature was indeed characteristic of the contraction induced by bile salt.

In the case of leech muscle, strong contraction was obtained with 15 mgm. of bile salt in 7 cc. of Ringer's solution, an amount definitely above "threshold" level.

2. *Bile salt does not enhance response to acetylcholine.* From the first demonstration by Loewi and collaborators (Loewi and Navratil, 1926; Engelhart and Loewi, 1930) it has been known that the optimum response of isolated muscle to acetylcholine requires prior treatment with physostigmine. The latter substance prevents rapid inactivation of the acetylcholine by the blood or tissue esterase. We made a systematic search for a similar effect of bile salt, with entirely negative results. Different concentrations of the latter agent in Ringer's solution proved completely devoid of the characteristic eserine effect. Even the large amounts that in themselves induced muscular contraction gave no greater contraction when acetylcholine was placed in the Ringer's fluid.

3. *Bile salt does not preserve acetylcholine in whole blood.* In our final series of experiments we compared the effect of prostigmine with that of bile salt on preservation of acetylcholine added to freshly drawn dog and cat blood. In every experiment two samples of blood were prepared; each sample contained 4 cc. of blood plus 3 cc. of Ringer's solution. One sample contained, in addition, $\frac{1}{4}$ mgm. of prostigmine plus acetylcholine in a concentration of 1:3,000,000. A second sample contained bile salt (5 mgm.) plus the same amount of acetylcholine, but no prostigmine. These two samples were allowed to stand at room temperature for an average time of four hours, then tested on the frog rectus muscle. At the end of the time stated, the sample with prostigmine contained 100 per cent of the original acetylcholine; the sample with the bile salt contained a negligible amount. There was therefore complete absence of esterase inactivation by the amount of bile salt used. In several control determinations we added acetylcholine to whole blood, waited some hours for the acetylcholine

to disappear as the result of inherent esterase activity, then added prostigmine or bile salt just prior to assay. In these latter cases the effect on the muscle was always negligible. The above observations prove that the esterase activity of whole blood is not appreciably inhibited by bile salt. Typical experimental results comparing the acetylcholine preserving power of bile salt with that of prostigmine are shown in figure 3.
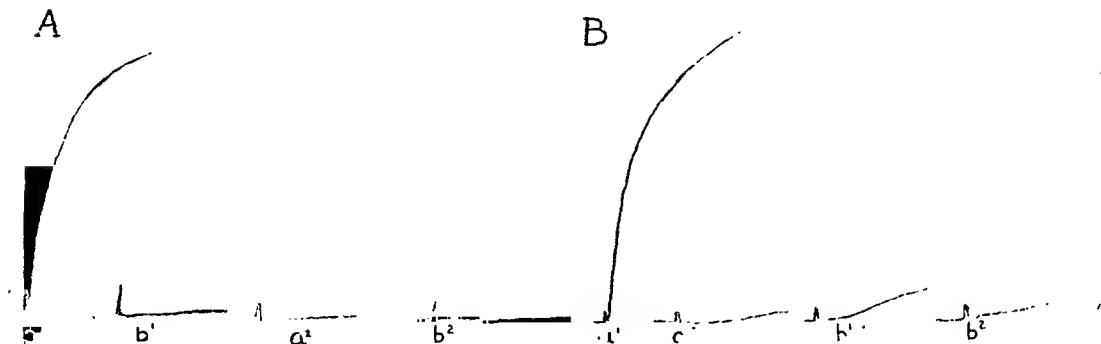
Fig. 3 compares the acetylcholine preserving power of bile salt with that of prostigmine as shown by effects on the frog rectus abdominis muscle. First, a known amount of acetylcholine was added to dog's blood (A) and to cat's blood (B). In both sets of curves a^1 represents the contraction of the rectus muscle four hours later when $\frac{1}{4}$ mgm. prostigmine had been added; all of the original acetylcholine is still present. b^1 shows complete disappearance of the acetylcholine in the same blood in the presence of 5 mgm. bile salt instead of prostigmine. a^2 and b^2 are control curves in which the prostigmine and bile salt respectively were added just before the assay to blood which had stood for four hours.

SUMMARY AND CONCLUSIONS

1. Bile salt in moderate doses (maximum 100 mgm. for a cat, 300 mgm. for a dog, injected intravenously) produces a consistent, though short-lasting fall of the blood pressure. This depressor action persists after atropine, after vagotomy, and after destruction of the spinal cord, and is of peripheral origin.

2. The hypotensive action of bile salt is only occasionally accompanied by cardiac slowing. This slowing is abolished by vagotomy and by atropine, and is therefore of central vagus origin.

3. Bile salt by itself, unlike prostigmine, does not enhance acetylcholine action; nor is its own action influenced by prostigmine. The characteristic depressor effect of bile salt in the circulating blood is therefore not essentially due to inhibition of cholinesterase.

4. These conclusions do not support the main contention of Antopol et al. (loc. cit.) or of Sobotka and Antopol (loc. cit.) regarding the physiological mechanism of the action of bile salt, nor the practical inference that the "vagotonic" signs of biliary disease are due to esterase inhibition.

from the Associate Committee on Medical Research of the National Research Council of Canada, to whom our thanks are due.

REFERENCES

ANTOPOL, W., L. TUCHMAN AND A. SCHIFRIN. Proc. Soc. Exper. Biol. and Med. **36:** 46, 1937.

ANTOPOL, W., A. SCHIFRIN AND L. TUCHMAN. Proc. Soc. Exper. Biol. and Med. **38:** 363, 1938.

BALTACEANO, G. AND C. VASILIU. C. R. Soc. de Biol. **115:** 1550, 1934; ibid. **115:** 1552, 1934; ibid. **116:** 550, 1934; ibid. **116:** 548, 1934.

ENGELHART, E. AND O. LOEWI. Arch. f. exper. Path. u. Pharmakol. **150:** 1, 1930.

HORRALL, O. H. AND A. J. CARLSON. This Journal **85:** 591, 1928.

LANDOIS. Deutsch. Klinik **46:** 449, 1863. (Quoted by HORRALL and CARLSON, *loc. cit.*)

LOEWI, O. AND E. NAVRATIL. Pflüger's Arch. **214:** 689, 1926.

LÖWIT. Ztschr. f. Heilk. 1882, p. 459. (Quoted by MEYER and GOTTLIEB, Experimental pharmacology. Lippincott, Philadelphia, 1926.)

McARDLE, B. Quart. J. Med. **9:** 107, 1940.

MELTZER, S. J. AND W. SALANT. J. Exper. Med. **7:** 280, 1905.

SOBOTKA, H. AND W. ANTOPOL. Enzymologia **4:** 189, 1937.

SOLLMANN, T. A manual of pharmacology. W. B. Saunders Co., Philadelphia and London 6th ed., 1942, pp. 212-214.

STILL, E. U. This Journal **88:** 729, 1929.

WAKIM, K., H. E. ESSEX AND F. C. MANN. Am. Heart J. **18:** 171, 1939.

WEINTRAUD. Arch. exper. Path. u. Pharmakol. **34:** 37, 1894. (Quoted by MEYER and GOTTLIEB, Experimental pharmacology, Lippincott, Philadelphia, 1926.)

THE RÔLE OF THE ADRENAL CORTEX IN ANOXIA: THE EFFECT OF REPEATED DAILY EXPOSURES TO REDUCED OXYGEN PRESSURE

GEORGE W. THORN, BENJAMIN F. JONES, ROGER A. LEWIS,[1] ELLIS R. MITCHELL[2] AND GEORGE F. KOEPF[3]

With the assistance of THOMAS J. KENNEDY, JR., HENRY M. GELFAND[4] and HARRY EISENBERG

From the Chemical Division, Medical Clinic, The Johns Hopkins University and Hospital, Baltimore, Maryland, and the Division of Industrial Hygiene, National Institute of Health, U. S. Public Health Service, Bethesda, Maryland

Armstrong and Heim (1, 2) first called attention to changes which occurred in the adrenal glands of rabbits exposed repeatedly to low pressure. In their experiments young male rabbits were exposed for four hours daily, five days each week, in a decompression chamber to a pressure equivalent to 18,000 feet altitude. We have attempted to confirm the findings of Armstrong and Heim with respect to the changes in the adrenal glands of animals so exposed and have, in addition, extended the studies to include observations on other physiological and biochemical changes which occurred in animals subjected to this new environment.

I. STUDIES ON RATS. A. *Methods.* Young male rats of the Sprague Dawley strain weighing approximately 150 grams were used in all experiments. During the period of exposure the diet consisted exclusively of Purina Dog Chow. The control animals received the same diet and care as the exposed animals. During the periods in which the exposures to decreased pressure were being made, the control animals were placed in a cage outside of the decompression chamber in the same room. The temperature in the room was approximately the same as that within the decompression chamber.

A small metal decompression chamber equipped with an electric light and a glass window was used in these studies. The ventilation rate through the small chamber varied between 8 liters per minute at a pressure of 226 mm. Hg and 75 liters per minute at a pressure of 429 mm. Hg. The temperature within the chamber remained relatively constant, i.e., 23°C. $\pm$ 2.5°. Exposures were made for four hours daily, five days a week for five to eight weeks. No exposures were made on Saturdays or Sundays.

One group of rats was exposed to a normal atmosphere at a barometric pressure of approximately 379 mm. Hg (pO$_2$ c. 80 mm. Hg). According to the

---

[1] John D. Archbold Fellow in Medicine, Johns Hopkins University

[2] Junior Chemist, Division of Industrial Hygiene, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

[3] John H. Harris Fellow in Medicine, Johns Hopkins University.

[4] Under Scientific Aide, Division of Industrial Hygiene, National Institute of Health U. S. Public Health Service, Bethesda, Md.

Altitude-Pressure Tables Based on the United States Standard Atmosphere (3) this is approximately equivalent to the pressure at an altitude of 18,000 feet. Henceforth in this discussion the pressure levels employed for exposure will be referred to in terms of equivalent altitude expressed in feet using the data of Altitude-Pressure Tables Based on the United States Standard Atmosphere and unless specifically stated no corrections will be made for temperature within the exposure chamber.   Another group of animals was exposed to a barometric pressure of 258 mm. Hg, (pO_2 c. 54 mm. Hg) equivalent to 27,000 feet altitude. Individual weight records were kept in all instances.   Following the completion of a series of exposures the animals were rested for 48 hours and sacrificed after a 24 hour fast.   At this time the blood sugar (4) and liver glycogen content (5) were determined and in a few animals red blood cell volume (6) was measured. Complete post-mortem examinations were made and microscopic sections of the adrenals, thyroids and hypophyses were studied.

Adrenalectomized rats were also exposed to reduced pressure.   These animals were maintained by means of 1 per cent sodium chloride in the drinking water, or daily injections of adrenal cortical extract[5], or a single pellet of crystalline desoxycorticosterone acetate[6] (125 mgm.) implanted subcutaneously.

B. *Observations.*   Normal young male rats were able to withstand repeated exposures of four hours daily in the decompression chamber at a pressure equivalent to 18,000 feet altitude.   The majority of animals were also able to withstand repeated exposures at a pressure equivalent to 27,000 feet altitude.   It is interesting to note that in most instances the animals that did not survive exposure at the higher altitude (27,000 ft.) died during or following the *first* or *second exposure* period, and not as a consequence of repeated exposures.

In general the animals showed greater restlessness, a higher rate of respiration, evidence of air hunger and signs of irritability and discomfort during the first exposure.   Animals that exhibited a great degree of motor activity in the chamber usually tolerated the exposures poorly.   Animals that survived repeated exposures were observed to rest quietly during the exposure and to recover rather rapidly after recompression to atmospheric pressure.

Repeated daily exposures for five successive days in each week at a pressure equivalent to 18,000 feet altitude had little or no effect upon the rate of growth of young rats (fig. 1).   At a pressure equivalent to 27,000 feet altitude, however, there was a noticeable slowing in the rate of weight gain *during periods of exposure* (fig. 2).   These periods of decreased rate of growth were followed by periods of accelerated rate of weight gain during the non-exposure periods (48 hrs. each week, i.e., Saturdays and Sundays).

The hematocrit (per cent red cell volume) was determined in three rats which had been exposed on 27 occasions to a pressure equivalent to 27,000 feet altitude.

---

[5] The adrenal cortical extract used in this study was provided by Dr. E. C. Kendall of the Mayo Clinic, Rochester, Minn.

[6] Crystalline desoxycorticosterone acetate (pellets of 125 mgm. each) and desoxycorticosterone acetate in sesame oil (Percorten) were provided through the courtesy of Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., Summit, N. J.

The average value for the hematocrit was 80 in the exposed animals as compared to 60 in unexposed controls. These observations are, of course, too few to be conclusive but suggest that repeated, short-period exposures to low pressure have an effect on hematopoiesis similar to that which has been observed in human subjects and certain animals during continued residence at high altitudes.
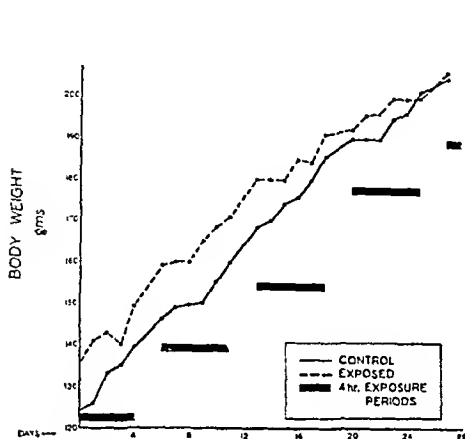
Fig. 1   Fig. 2
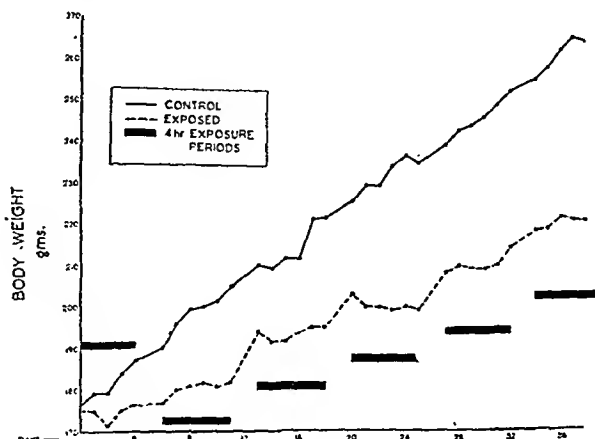
Fig. 1. The effect of repeated daily exposures, 4 hours daily 5 days a week, to a pressure equivalent to 18,000 feet altitude on the rate of growth of young male rats.

Fig. 2. The effect of repeated daily exposures, 4 hours daily 5 days a week, to a pressure equivalent to 27,000 feet altitude on the rate of growth of young male rats.
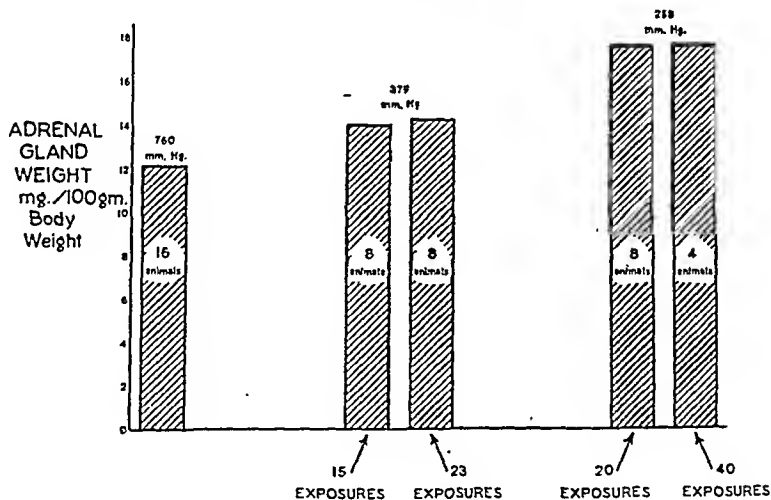
Fig. 3. The effect of repeated exposures to low pressure on adrenal gland weight of young male rats.

A striking change was noted in the size of the adrenal glands (fig. 3) of all animals exposed repeatedly to a pressure equivalent to either 18,000 or 27,000 feet altitude. The change, however, was more striking in the animals which had been exposed to the pressure equivalent to the higher altitude (27,000 ft.). The degree of pressure reduction appeared to be a more important factor than the number of exposures (fig. 3).

The principal change seen in the adrenals of the exposed rats was a thickening of the cortex which accounts for the greater part of the enlargement of the gland. Part of this enlargement was due to a marked dilatation of the capillary bed, particularly in the inner layer of the cortex and in the medulla. There was an increase in the amount of fat in the cells of the zona fasciculata, most marked in the peripheral portion. However, the greatest and most consistent anatomical change was a thickening of the zona reticulata due to an apparent increase in the number of cells. The cells of this zone are thought by Bennett (7) to represent the end-stage of secretory activity.

TABLE 1

Effect of repeated exposures to a pressure of 258 mm. Hg (27,000 ft.) on the blood glucose of 24 hour fasted rats (4-8 weeks' exposure)

GROUP	NUMBER OF RATS	BLOOD GLUCOSE
		mm. per 100 cc.
Control..............................	4	70
Exposed..............................	8	59

TABLE 2

The relative effectiveness of specific therapy in preventing the death of adrenalectomized rats exposed repeatedly to low pressure (4 hrs. daily, 5 days a wk., 4 wks.)

THERAPY	NUMBER OF ANIMALS	MORTALITY	PER CENT MORTALITY
A. Pressure level 379 mm. Hg (18,000 ft., altitude equivalent)			
			per cent
Sodium chloride.........................	4	4	100
Desoxycorticosterone acetate.............	4	0	0
Adrenal cortical extract.................	4	0	0
Normal controls........................	20	0	0
B. Pressure level 258 mm. Hg (27,000 ft., altitude equivalent)			
Sodium chloride.........................	4	4	100
Desoxycorticosterone acetate.............	4	2	50
Adrenal cortical extract.................	4	0	0
Normal controls........................	16	0	0

The medulla was either normal or slightly increased in size in the adrenal glands of exposed animals.

A study of the blood sugar levels in fasted, normal rats (table 1) indicated that following repeated exposures to low barometric pressure these values were somewhat lower than those observed in control, unexposed animals. Liver glycogen values in both groups of fasting animals (exposed and unexposed) were very low. These observations are of particular interest in contrast to the very high carbohydrate levels which were observed in normal rats exposed to anoxia for a single period of 24 hours (8, 9).

Adrenalectomized rats. Adrenalectomized rats, maintained with sodium chlo-

ride, were unable to survive repeated exposures to a pressure equivalent to 18,000 feet or 27,000 feet altitude (table 2). Animals treated with pellets of synthetic desoxycorticosterone acetate were able to survive repeated exposures to a pressure equivalent to 18,000 feet altitude but were unable to survive (50 per cent mortality in a group of 4 animals) repeated exposures to a pressure equivalent to 27,000 feet altitude. Daily injections of 1 cc. of adrenal cortical extract (Kendall) enabled adrenalectomized rats to survive repeated exposures to a pressure equivalent to either 18,000 feet or 27,000 feet altitude (table 2).

DISCUSSION. It is of considerable interest to note that although young male rats readily survived repeated exposures of 4 hours daily at a pressure equivalent to 27,000 feet altitude, a marked decrease in the rate of growth occurred during the days on which the exposures were made. In contrast, the rate of growth was accelerated during the two-day rest period (Saturday and Sunday) each week. It is probable that the delay in growth may have been occasioned by a reduction in appetite and food intake. This question cannot be answered at present since the food intake of the exposed animals was not measured accurately. The decreased rate of growth which was observed in the exposed animals should provide an excellent objective test method for studying the efficacy of various forms of therapy on improving adaptation to repeated exposures to low pressure.

The increased size of the adrenals which occurred in rats exposed repeatedly to low pressure supports the observations of Armstrong and Heim (1) on rabbits. The extent to which pressure was reduced appeared to be a more important factor than the total number of exposures in increasing the size of the adrenals.

The marked increase in red blood cells which occurred during the relatively short periods of exposure to reduced pressure is of special interest. These changes suggest that it is not necessary to reside continuously at high altitude in order to obtain a marked increase in red blood cell count.

Earlier studies (Evans, 8; Lewis, Thorn et al., 9) indicated that exposure to anoxia for a period of 24 hours was attended by a striking rise in carbohydrate levels. Lewis, Thorn et al. (9) further demonstrated that this latter phase of carbohydrate plethora followed an initial phase (2-6 hrs.) during which there was a marked depletion of carbohydrate reserves. It appears from the present studies that repeated exposures to reduced barometric pressure do not result in the accumulation of carbohydrate reserves which occurs following a single 24-hour exposure to anoxia, but rather results in an appreciable reduction in carbohydrate stores.

The effect of specific therapy on the survival of adrenalectomized rats exposed to decreased pressure indicated that sodium chloride treatment offered relatively little protection, synthetic desoxycorticosterone acetate offered partial protection and adrenal cortical extract more complete protection.

II. STUDIES ON RABBITS. A. *Methods.* Young male albino rabbits, weighing approximately 2 kgm. and approximately 3 months of age, were used in these experiments. During the period of exposure the diet consisted exclusively of rabbit chow (Maritime Milling Co.). Control animals received the same diet and general care as the exposed animals.

Rabbits were exposed in a small decompression chamber identical with that described in the experiments on rats (see Methods). Exposures were made for four hours daily *seven days a week* to a normal atmosphere at a barometric pressure of 379 mm. Hg, in which the partial pressure of oxygen was 80 mm. Hg. According to the U. S. Standard atmosphere pressure this is equivalent to 18,000 feet altitude. Another group of animals was exposed to a pressure level of 282 mm. Hg, in which the partial pressure of oxygen was 59 mm. Hg, equivalent to 25,000 feet altitude.

Individual weight records were kept on all animals. Following the completion of a series of exposures, the animals were permitted to rest over night under fasting conditions and on the following morning a specimen of heart's blood was obtained under oil after which the animals were sacrificed. Studies were made of the blood sugar, non-protein nitrogen, serum sodium, chloride, carbon-dioxide combining power, potassium, and red blood cell volume. Com-

TABLE 3

Survival of normal rabbits exposed to low pressure: exposed for 4 hours daily, 7 days a week

PRESSURE	NUMBER OF ANIMALS	TOTAL NUMBER OF EXPOSURES	NUMBER OF ANIMALS SURVIVING	PER CENT SURVIVAL
mm. Hg				
Control	9		9	100
379	5	35	5	100
282	15	21	5	33
282	31	6	9	29

TABLE 4

Effect of repeated exposures to low pressure on hematocrit and oxygen content of blood of normal rabbits: exposed for 4 hours daily, 7 days a week

PRESSURE	NUMBER OF ANIMALS	TOTAL NUMBER OF EXPOSURES	O₂ PER 100 CC. OF BLOOD	HEMATOCRIT, VOL. PER CENT PACKED RBC.
mm. Hg			*cc.*	
Control	9		18.3	42.7
379	4	35	21.8	49.8
282	4	21	22.3	58.0

plete post-mortem examination was made and the weights of the thymus and adrenal glands were recorded.

B. *Observations.* Normal, young male rabbits were able to withstand repeated exposures of four hours daily, seven days a week, for five weeks, to a pressure equivalent to 18,000 feet altitude. No deaths occurred in this group of animals during the five weeks of exposure. However, in contrast to normal rats, many of the normal rabbits succumbed when exposed to a pressure equivalent to 25,000 feet altitude. In a group of 15 animals, 3 succumbed on the first day of exposure, 4 died on the second day, and one each on the fourth, fifth and twelfth days. This experiment was terminated at the end of 21 exposures (3 wks.) at which time only 5 animals had survived (table 3).

Post-mortem examination of the animals that succumbed at a pressure equivalent to 25,000 feet altitude showed hemorrhages into the lungs and not infrequently herniation of dilated loops of intestine through the diaphragm into the thoracic cavity.

To confirm the pathological changes that were observed in a group of 15 animals exposed to a pressure equivalent to 25,000 feet altitude, an additional group of 31 animals was exposed in the same way for 4 hours daily for 2 to 9 days. During this period there were 22 deaths, i.e., 4 on the first, 7 on the second, 3 on the third, 3 on the fourth, 4 on the fifth and 1 on the sixth day, respectively.

Post-mortem examinations revealed the following gross findings: 1. Pulmonary edema and hemorrhage in practically all cases. 2. In 9 cases evidence of diaphragmatic rupture and herniation of abdominal viscera into the thoracic cavity, with occasional rupture of the stomach.

Further evidence has been obtained which indicates that expansion of intestinal gases during altitude exposure is largely responsible for failure of rabbits
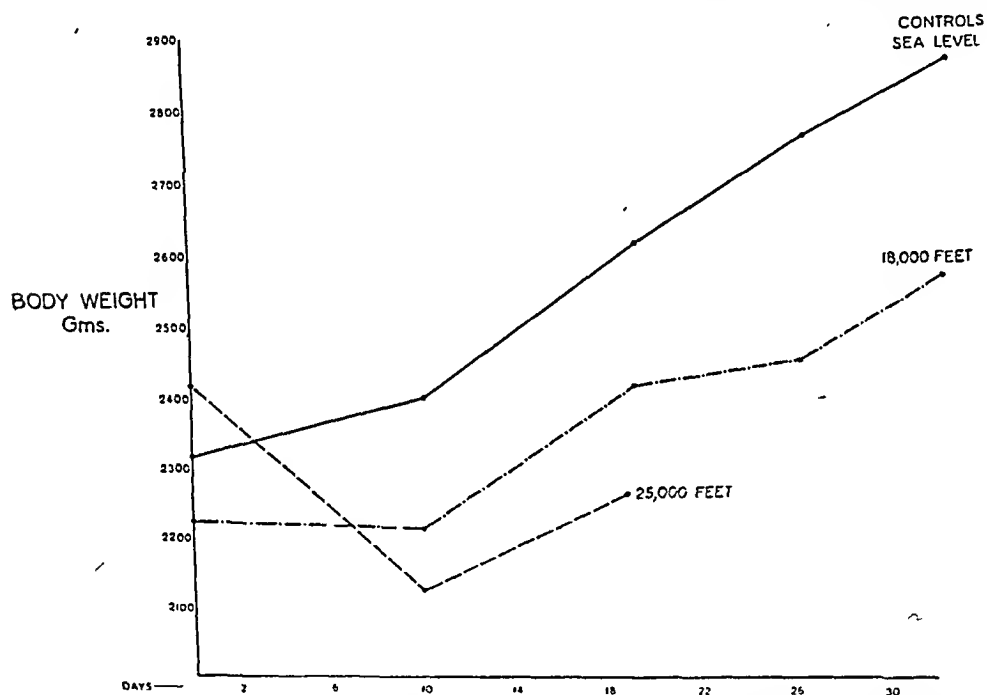
Fig. 4. The effect of repeated daily exposures to low pressure on the rate of growth of young male rabbits.

as a species to tolerate reduced pressures which are well tolerated by other rodents. Thus 4 rabbits which were fed a milk diet for 3 days instead of their usual bulky diet and 2 rabbits which were fed the normal diet to which activated charcoal had been added were able to survive four hour exposures to a pressure equivalent to 25,000 feet altitude, whereas one of two rabbits which were placed in an atmosphere of oxygen for 22 minutes and then decompressed to a pressure equivalent to 36,000 feet altitude *in an atmosphere of oxygen* died and revealed at post mortem the characteristic changes previously described.

Repeated exposures to low pressure had a pronounced effect on the weight gain of young male rabbits (fig. 4). Animals exposed to a pressure equivalent to 18,000 feet altitude failed to gain weight during the first 10 days of exposures

in contrast to control animals in which an average weight gain of approximately 100 grams was observed. However, after this initial period, the animals exposed to a pressure equivalent to 18,000 feet altitude gained weight at approximately the same rate as the control animals. Rabbits exposed to a pressure equivalent to 25,000 feet altitude lost weight during the first half of the exposure, and survivors thereafter showed an increase in weight.

A consistent increase in oxygen capacity of the blood and in per cent red blood cell volume (hematocrit) was noted in rabbits exposed repeatedly to low pressure (table 4). The increase in hematocrit values and oxygen capacity of the blood was somewhat greater in the group of animals which had been exposed to the lower pressure, i.e., equivalent to 25,000 feet altitude. In these experiments a study of the serum electrolyte concentration indicated that in the group of animals exposed to a pressure equivalent to 25,000 feet altitude there was a
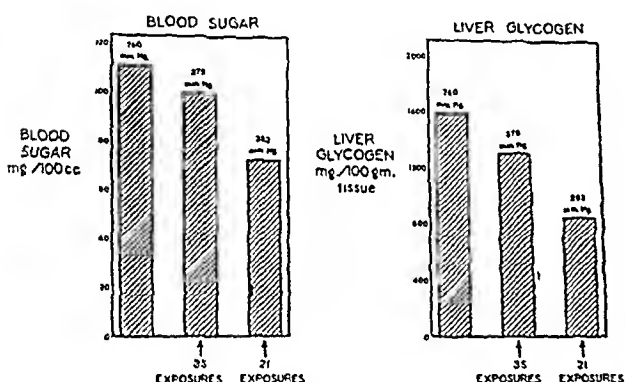
Fig. 5
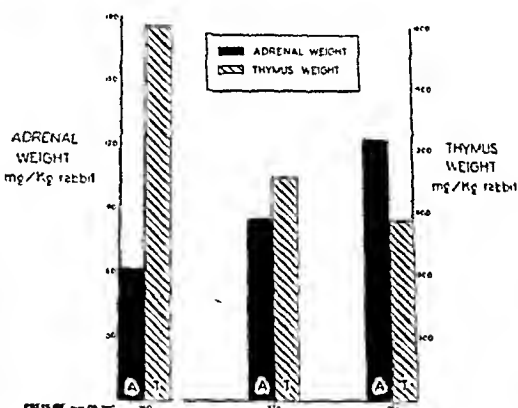
Fig. 6

Fig. 5. The effect of repeated exposures to low pressure on blood sugar and liver glycogen levels in normal rabbits.

Fig. 6. The effect of repeated daily exposures to low pressure on adrenal and thymus weights in normal rabbits.

lowering of serum sodium (6 m.eq.), chloride (4 m.eq.) and carbon dioxide-combining power (2.4 m.eq.) and a slight rise in serum potassium (0.7 m.eq.) and blood non-protein nitrogen (8 mgm.). A decrease in serum sodium (5 m.eq.) and a slight rise in blood non-protein nitrogen were the only changes which were observed in the group of animals exposed repeatedly to a pressure equivalent to 18,000 feet altitude.

The effect of repeated exposures to low pressure on blood glucose and liver glycogen levels was also studied. The observations made in these experiments (8 animals in each group) suggest that repeated exposures to low pressure resulted in lowered carbohydrate levels (fig. 5). The depletion of carbohydrate reserves was much greater in the animals which had been exposed to the lower pressure (25,000 ft.). It is of interest to contrast the depletion of carbohydrate reserves which occurred in animals exposed repeatedly to anoxia with the great

increase in carbohydrate levels which was observed in rabbits following a single 24-hour exposure to anoxia (Lewis, Thorn et al., 9).

The most striking changes which occurred in these experiments was the increase in adrenal gland weight and the decrease in thymus weight (fig. 6). The increase in adrenal gland weight is similar to that which has been described by Armstrong and Heim (1, 2) and which we also observed in rats. The decrease in thymus weight has not been reported previously in animals exposed to low barometric pressure although a similar decrease in thymus weight has been observed in normal animals treated with excessive quantities of certain adrenal steroids (10).

DISCUSSION. A strict comparison between the results obtained in this series of experiments and those obtained by Armstrong and Heim (1, 2) is not possible since these investigators exposed rabbits for 4 hours daily, 5 days each week and subjected the animals to an "altitude tolerance test" once during the two days in each week when regular exposures were not made. Although the "altitude tolerance test" was not fatal when employed by these investigators on control unexposed rabbits, all the fatalities in the exposed group of animals occurred during the weekly altitude tolerance test and not during the daily exposure period at a pressure equivalent to 11,000 or 18,000 feet altitude. In the group exposed at 18,000 feet altitude the mortality rate was approximately 75 per cent in the experiments of Armstrong and Heim.

Armstrong and Heim concluded that daily exposures were followed by improved adaptation for a period of 3–4 weeks after which deterioration occurred. In a second series of experiments they followed changes in weight, mortality, blood cytology and blood non-protein nitrogen in a group of rabbits exposed daily at a pressure equivalent to 18,000 feet altitude, 5 days a week. These animals were also subjected to an altitude tolerance test. They observed a gradual decline in body weight and in hemoglobin in these animals associated with a decrease in blood non-protein nitrogen. These changes were accompanied by decreased altitude tolerance. However, their figures (2) as given do not support the hypothesis that the mortality rate increased after repeated exposures since the weekly death rate of the 32 animals was 0, 1, 3, 2 and 0 in the 1st, 2nd, 3rd, 4th and 5th weeks respectively.

Our results differed considerably from those of Armstrong and Heim since at a pressure equivalent to 18,000 feet altitude the animals showed a gradual adaptation accompanied by an improvement in general reaction, gain in weight and by a rise in hematocrit values although there was an appreciable reduction in concentration of base in the serum (approximately 6 m.eq.).

Concerning the mortality rate of rabbits exposed repeatedly to low pressure we have had the same experience as Armstrong, but have not subjected the animals to the rigorous altitude tolerance test, thus avoiding fatalities at altitudes of 18,000 feet and below. At a pressure equivalent to 25,000 feet altitude the mortality rate was very high (approximately 75 per cent) in our series of experiments.

Post-mortem study of the rabbits that succumbed revealed areas of atelectasis and hemorrhage described by Armstrong and Heim (2). In addition to this it was noted that in many instances rupture of the diaphragm, or stomach, or both had occurred.

A careful study of the organs of the rabbits that had survived the 18,000 feet exposure revealed that the adrenals had enlarged, as Armstrong and Heim (2) have shown, and furthermore that the thymus had undergone atrophic change. The reciprocal relationship between adrenal cortex and thymus is well known (10, 11).

From these studies it is apparent that the rabbit is an unsuitable species for investigating the effects of anoxia produced by low barometric pressure because of mechanical disturbances which result from expansion of intestinal gases. However, rabbits, like rats, appear to adapt themselves readily to repeated exposures to a moderate reduction in barometric pressure (18,000 ft. altitude). This adaptation is accompanied by an increase in hemoglobin, an enlargement of the adrenal glands and a striking decrease in the weight of the thymus.

III. STUDIES ON DOGS. A. *Methods.* Adult, male dogs, weighing 10 to 15 kgm., were used in these experiments. The animals were maintained on a diet which consisted of raw beef. They were fasted for 16 hours before each exposure and were fed approximately 2 hours after the end of the exposure period. The care of the animals and the technics used in these studies have been described (9). Careful weight records were kept.

The animals were exposed in a large decompression chamber equipped with electric light and plate glass observation windows. The ventilation rate through the chamber was 50 to 60 cubic feet per minute. The temperature within the chamber remained relatively constant, i.e., 23° ± 2°C. It was possible to expose 4 dogs in individual cages simultaneously. Exposures were made for four hours daily, five days a week at a barometric pressure level of 282 mm. Hg, in which the partial pressure of oxygen was 59 mm. Hg (equivalent to 25,000 ft. altitude). No exposures were made on Saturdays or Sundays.

Two bilaterally adrenalectomized dogs, maintained by means of subcutaneously implanted pellets of crystalline desoxycorticosterone acetate, were also subjected to repeated exposures to low barometric pressure. Prior to exposure, these animals were in good physical condition and chemical studies of the blood revealed no abnormalities.

B. *Observations.* Normal dogs exposed to a barometric pressure of 282 mm. Hg (equivalent to 25,000 ft. altitude) for four hours daily, five days a week, soon lost appetite and their weight decreased. They were observed to vomit occasionally during exposure and frequently in the periods between exposures. The weight loss varied considerably in different animals (fig. 7). As far as could be detected normal dogs showed no other obvious signs and symptoms from repeated exposures at this pressure. It is interesting to note that the dogs did not regain their former weight for several months after the exposures had been discontinued. Adrenalectomized dogs, maintained on desoxycorticosterone
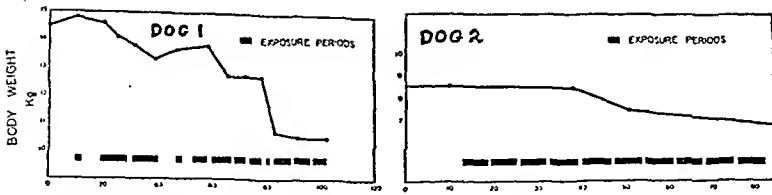
Fig. 7. The effect of repeated daily exposures to low pressure equivalent to 25,000 feet altitude on weight of normal dogs.
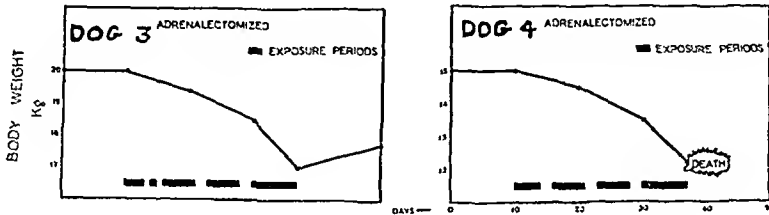
Fig. 8. The effect of repeated daily exposures to low pressure equivalent to 25,000 feet altitude on weight of adrenalectomized dogs maintained with synthetic desoxycorticosterone acetate.
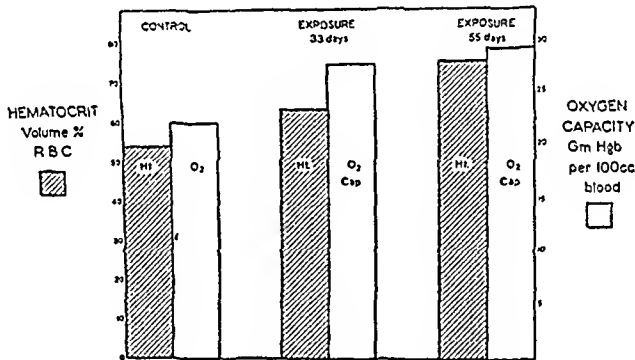
Fig. 9. The effect of repeated exposures to decreased pressure on hematocrit and oxygen capacity of blood-normal dogs.
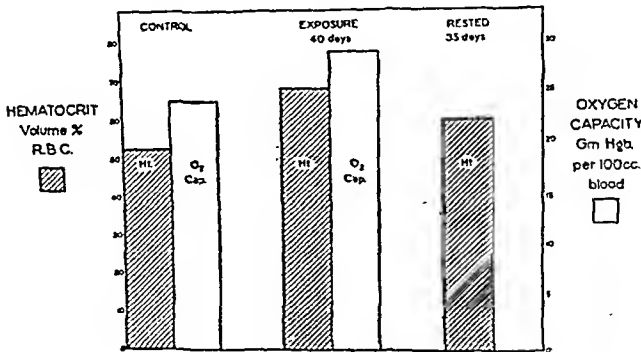
Fig. 10. The effect of repeated exposures to decreased pressure on hematocrit and oxygen capacity of blood-normal dogs.

acetate, were not able to withstand exposure to reduced barometric pressure equivalent to 25,000 feet altitude (fig. 8); exposure of dog 220 was discontinued because of the poor condition of this animal.

Blood chemical studies revealed no significant changes in the normal dogs exposed repeatedly to low barometric pressure (table 5). This is in marked contrast to the shift in serum chloride and carbon dioxide which was observed in

TABLE 5

Blood chemical findings in normal dogs exposed repeatedly to low barometric pressure: 4 hours daily, five days a week

PRESSURE	NUMBER OF ANIMALS	TOTAL NUMBER OF EXPOSURES	SERUM SODIUM	SERUM CHLORIDE	SERUM CO₂ COMBINING POWER	BLOOD NON-PROTEIN NITROGEN	SERUM PROTEIN
mm. Hg			*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*mgm./100 cc.*	*gm./100 cc.*
Control	3		147.2	108.8	24.6	35	6.2
282	3	22–66	146.2	109.5	24.8	44	6.3

TABLE 6

Changes in red blood cell count, hematocrit and oxygen capacity of the blood in normal dogs exposed repeatedly to low pressure: 4 hours daily, 5 days a week (25,000 ft. altitude pressure equivalent)

ANIMAL	NUMBER OF EXPOSURES	HEMATOCRIT, VOLUME PER CENT PACKED RED CELLS	RED BLOOD CELLS PER CU. MM.	OXYGEN CAPACITY, GM. HBG. PER 100 CC. BLOOD
5	Control period	54.0		22.4
	33 days exposure	63.2	12.0	27.9
	55 days exposure	75.0		29.3
6 .	Control period	55.5	9.0	24.9
	44 days exposure	68.6		29.0
	70 days rested	52.5		
1	Control period	52.6	9.5	24.3
	40 days exposed	68.2	13.2	29.2
	35 days rested	60.2		
7	Control period	56.0	9.2	23.4
	22 days exposure	61.5		
	50 days rested	55.0		
	18 days exposure	66.0		27.5

normal dogs exposed to the same decrease in oxygen tension for a single period of 24 hours.

The most striking change which was observed in these experiments was the extent to which the red blood cells and hemoglobin increased in dogs exposed repeatedly to low barometric pressure (table 6). In 4 animals prior to exposure the average packed red blood cell volume (hematocrit) was 54.5 per cent, the red blood cell count was 9.2 million and the oxygen capacity was 23.7 cc.

oxygen per 100 cc. blood. During exposure to low pressure for 18 to 55 days (39 day average) the red blood cell volume (hematocrit) increased to 69.4 per cent, the red blood cell count increased to 12.6 millions and the oxygen capacity rose to 28.4 cc. oxygen per 100 cc. blood. During rest periods of 35 to 70 days the average hematocrit value fell to 55.9 per cent (fig. 10).

SUMMARY

1. *Young male rats*, exposed repeatedly to a pressure equivalent to 18,000 feet altitude for four hours daily, five days a week over a period of 5 to 8 weeks, maintained a normal rate of growth and appeared to tolerate the exposures rather well. The majority of a group of young male rats, exposed in a similar manner to a pressure equivalent to 27,000 feet altitude tolerated the exposures but failed to gain weight at a normal rate during the days on which exposures were made. Adaptation to repeated short exposures to low pressure was associated with *a*, increase in hematocrit values (volume per cent of packed red blood cells); *b*, increased weight of adrenals. The fasting levels of blood sugar and liver glycogen of animals exposed repeatedly to low barometric pressure were normal or lower than normal. Adrenalectomized rats were unable to withstand repeated exposures to low barometric pressure unless treated with adrenal cortical hormone.

2. *Young male rabbits* tolerated repeated daily exposures (4 hrs., 7 days a wk., for 5 wks.) to reduced barometric pressure equivalent to 18,000 feet altitude. Such animals did not tolerate repeated exposures to a pressure equivalent to 25,000 feet altitude. Death of animals was associated with hemorrhages into the lungs and herniation of distended loops of intestine into the thoracic cavity. Most of the fatalities occurred during the first or second day of the exposure. Repeated exposures to low pressure equivalent to either 18,000 or 25,000 feet altitude were accompanied by a striking but temporary delay in rate of growth. A slight reduction in the concentration of sodium, chloride and in the carbon-dioxide combining power of the serum was noted in rabbits exposed repeatedly to a pressure equivalent to 25,000 feet altitude. The carbohydrate levels of rabbits exposed repeatedly to low pressure were reduced considerably below those of unexposed controls. Repeated exposures to low pressure were accompanied by *a*, increase in oxygen capacity of the blood; *b*, increase in hematocrit values (volume per cent of packed red blood cells); *c*, increase in adrenal weight; *d*, decrease in thymus weight.

3. *Normal dogs* exposed repeatedly to low barometric pressure equivalent to 25,000 feet altitude soon developed anorexia and weight loss. Normal weight was not restored for weeks after exposures had been discontinued. Adrenalectomized dogs maintained on desoxycorticosterone acetate did not tolerate repeated exposures to low barometric pressure equivalent to 25,000 feet altitude. No significant changes in serum electrolyte concentration were observed in dogs exposed repeatedly to low pressure. The great increase in the number of red blood cells, hematocrit value and oxygen capacity of the blood was the most striking change which was observed in normal dogs exposed repeatedly to low pressure.

REFERENCES

(1) ARMSTRONG, H. G. AND J. W. HEIM.   J. Aviation Med. **9**: 92, 1938.
(2) ARMSTRONG, H. G. AND J. W. HEIM.   J. Aviation Med. **9**: 45, 1938.
(3) BROMBACHER, W. G.   National Advisory Committee for Aeronautics, Report no. 538, 1935, reprinted 1942.
(4) FOLIN, O. AND H. MALMROS.   J. Biol. Chem. **83**: 115, 1929.
(5) GOOD, C. H., H. KRAMER AND M. SOMOGY.   J. Biol. Chem. **100**: 485, 1933.
(6) WINTROBE, M. M.   J. Lab. and Clin. Med. **15**: 287, 1929.
(7) BENNETT, H. S.   Am. J. Anatomy **67**: 151, 1940.
(8) EVANS, G.   This Journal **110**: 273, 1934.
(9) LEWIS, R. A., G. W. THORN, G. F. KOEPF AND S. S. DORRANCE.   J. Clin. Investigation **21**: 33, 1942.
(10) WELLS, B. B. AND E. C. KENDALL.   Proc. Staff Meetings of the Mayo Clinic **15**: 324, 1940.
(11) ROWNTREE, L. G. AND A. M. SNELL.   A clinical study of Addison's disease.   Mayo Clinic Monographs, Philadelphia, W. B. Saunders Co., p. 120.
(12) HALL, F. G., D. B. DILL AND E. S. GUZMAN BARRON.   J. Cell. and Comp. Physiol. **8**: 301, 1936.

THE DIFFERENTIAL EFFECTS OF RESPIRATION ON THE LEFT AND RIGHT VENTRICLES

ROBERT H. SHULER,[1] CHARLES ENSOR, R. E. GUNNING, W. G. MOSS AND VICTOR JOHNSON

From the Department of Physiology, University of Chicago

The systemic arterial blood pressure changes (fall in inspiration, rise in expiration) have commonly been thought to be due in part to fluctuations in the filling and consequently the output of the heart induced by respiratory fluctuations in intrathoracic pressure. The decreased intrathoracic pressure associated with inspiration is generally considered to have an "aspiratory" effect upon the veins within the thorax, and hence to augment the inflow of blood to the right heart (Burton-Opitz, 1902; Hooker, 1921; Wiggers, 1921; Visscher, Rupp and Scott, 1924; Heinbecker, 1927). Closed chest measurements of cardiac output by means of a glass oncometer similar to that first described by Wiggers and Katz (1922) have given rather contradictory results. Eyster and Hicks (1933) found that inspiration was associated with greater diastolic size, but with a decreased stroke volume, which seemed inconsistent with the Starling principle. Cahoon, Michael and Johnson (1941) found that there were both decreased diastolic size and decreased stroke volume during inspiration. These findings were consistent with the Starling principle, and seemed to account, in part, for the fall in arterial pressure during inspiration.

Boyd and Patras (1941), recognizing the fact that previous measurements of cardiac output had been made with the ventricles operating against an unchanging atmospheric pressure, designed a recording system wherein the ventricles operated against fluctuating negative pressures approximating those of the closed chest of a dog breathing normally. Their experiments showed that when the heart operated against approximately intrathoracic pressures instead of atmospheric pressures there was a definite increase of both diastolic volume and stroke volume during inspiration. These results were consistent with both the Starling principle and the concept of aspiration of blood into the thorax and heart in inspiration, but left unanswered this question: if the heart pumps more blood during inspiration, why should the arterial blood pressure fall during that phase of respiration?

Experiments employing the differential manometer. In an attempt to check the findings of Boyd and Patras, a recording system was designed which allowed free interchange of pressure variations between the interior of the chest and the ventricles in the oncometer, and which in addition eliminated the possibility of any respiratory displacement of the recording membrane. The method of recording was adapted to the stationary optical manometer devised by Hamilton, Brewer and Brotman (1934). The manometer was of the differential

---

[1] Now at University of Georgia Medical School, Augusta.

type, equipped with a very sensitive rubber membrane (see fig. 1, right).   One chamber of the manometer was connected to the oncometer, the other to the intrapleural space.   A communication was established between the two tubes leading to the chest and the oncometer respectively.   This communicating tube could be constricted or enlarged at will, thus affording a means of regulating the excursion of the recording membrane.   (The oncometer system was also con-
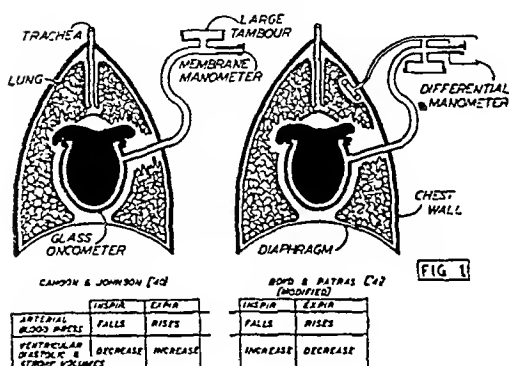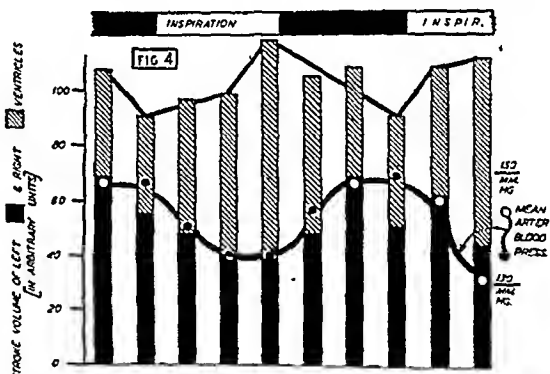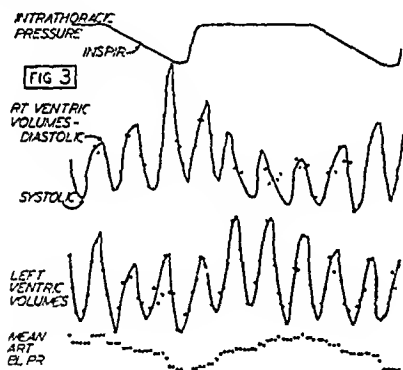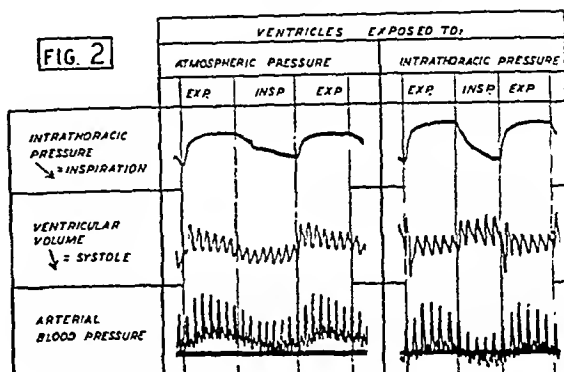
Fig. 1. Diagrammatic representation of cardiometer recording system.   At left is system used by Cahoon, Michael and Johnson; at right is system described in this paper.

Fig. 2. Simultaneous curves of intrathoracic pressure, ventricular volume, and carotid pressure.   At left with the ventricles under atmospheric pressure, at right with ventricles under intrathoracic pressure.

Fig. 3. Graph of intrathoracic pressure, right and left ventricular "volumes," and carotid pressure.   Ordinates for ventricular volumes are measured areas in square inches, abscissas are $\frac{1}{32}$ second intervals (movies at 32/sec.).

Fig. 4. Block diagram of same record as figure 3, showing right, left, and total ventricular stroke volumes in ten successive heart beats occurring during nearly two respiratory cycles. Mean carotid blood pressure is superimposed.

nected to the usual large closed air space which allowed a minimum of interference with cardiac filling and ejection.)   It can be seen that this system allowed free interchange of pressure variations between the chest and the oncometer, and that at the same time the influence of intrathoracic pressure was equal on both sides of the recording membrane.   By sufficiently constricting the intercommunicating tube, a condition could be obtained in which the relatively slow

chest pressure changes were distributed throughout the whole system, while the rapid movements of the heart could still cause excursions of the manometer membrane. Controls run during each experiment showed that there was no displacement of the membrane due to respiratory pressure changes. Recording could be done under the conditions used by Cahoon, Michael and Johnson (1941) (fig. 1, left) by closing the intercommunicating and intrapleural tubes. Direct comparisons of the two methods could be made from these records.

Medium-sized dogs anesthetized with sodium barbital were used. Artificial respiration was supplied by means of interrupted blasts, the 4th and 5th ribs on the right were resected, the pericardium removed, and an oncometer was placed over the two ventricles. Care was taken to assure that there was neither leakage nor constriction at the A-V groove. Before closing the chest, two metal trocars were inserted between the 2nd and 3rd ribs, one on either side of the midline. One of these trocars was attached directly to an optical manometer for recording intrathoracic pressure changes, and provided with a side tube through which air could be exhausted from the chest. The other trocar and the oncometer were attached to the differential manometer as described in the preceding paragraph. The chest was closed with large hemostats, the air was aspirated from the chest, and normal respiration allowed to proceed. Carotid pressures were recorded simultaneously.

Figure 2 shows records taken under the two different recording conditions, within approximately thirty seconds of each other. These results (right half of figure) confirm those of Boyd and Patras (1941) even to the point that the largest systolic excursion occurred when expiration and systole began simultaneously. No accurate calibration of the volume changes of the heart was obtainable with this recording system although a very crude calibration could be made at the close of the experiment by rapidly injecting into or removing from the oncometer tubing, known volumes of air.

Figure 2 demonstrates that carotid blood pressure dropped during inspiration whether the ventricles were exposed to atmospheric or intrathoracic pressure. In these and all previous records made with the ventricles under atmospheric pressure (fig. 2, left) the rise and fall in carotid pressure corresponded to the rise and fall of ventricular diastolic volume and stroke. But if the results obtained with the ventricles under intrathoracic pressure (fig. 2, right) are valid, it becomes necessary to explain why in inspiration the carotid pressure falls when the ventricles are ejecting more blood. It was thought that perhaps at the time of inspiration there might be an *increase* in the diastolic and stroke volumes of the *right* ventricle which would be so large as to mask a possible *decrease* in the *left* heart volume and stroke. Since no satisfactory method has been evolved to measure directly the volume changes of the ventricles independently of each other, the following experiment was devised:

Differential ventricular volumes. Large dogs, (18-25 kgm.) were used, anesthetized with sodium barbital. A ventral "plate" of ribs and muscle was removed from directly over the heart. A crucial incision was made in the pericardium and the edges were sutured back to the chest wall to form a cradle for the heart.

Small discs of white cardboard with black centers were affixed to the ventricles with collodion to outline each ventricle as completely as possible. After inserting a trocar through the chest wall for recording of respiration, a curved window of transparent plastic was placed over the chest opening, and the skin pulled up around it to form an air tight seal. The chest was evacuated and normal respiration allowed to proceed. Indicators, for recording carotid and intrathoracic pressures, were arranged to move above suitable scales within the optical field of a camera aimed at the heart. High speed motion pictures were taken under different experimental conditions.

Individual photographic enlargements were then made of a number of successive frames from each experiment. Each group of approximately 140 frames included at least two respiratory cycles. Lines were drawn between the markers on each print to outline each ventricle separately, using the intraventricular septum as common to both ventricles. The enclosed areas in each frame were measured with a planimeter and simultaneous blood and intrathoracic pressures observed. Only groups of frames were used where careful counts showed the heart rate to be constant. Figure 5 shows four frames representative of the comparative ventricular areas at the peak of systole and diastole during the height of inspiration and expiration respectively[2].

It must be remembered that it *is not* assumed that the measured areas are accurate reflections of the volumes of the ventricles, but to avoid coining new and useless names the terms "volume", "stroke" and "output" will be applied without modification. All numerical results are in arbitrary units, but they are strictly comparable in each experiment, since projection distances were kept constant throughout. The measured areas of the right and left ventricle were never more than approximately equal, but it was assumed that the output of the left ventricle would be equal to that of the right over a period of several respirations, and numerical results were in some cases corrected and graphing was done accordingly. However, the graphs showed the same qualitative results whether crude or corrected data were used.

Figure 3 gives the results of one experiment in which are plotted the measurements, from the successive movie frames, of left and right ventricular "volumes," intrathoracic pressure, and mean arterial blood pressure. It can be seen from this and the numerical data given in figure 5 that during inspiration the diastolic and stroke volumes in the right ventricle increase, while the corresponding measurements of the left ventricle decrease. The reverse is true during expiration. This corresponds with the findings of Cahoon, Michael and Johnson (1941) on the auricles: in inspiration the diastolic volume of the right auricle increases, while that of the left auricle decreases. Figure 4 shows that when measurements are made with the ventricles under intrathoracic pressure, the total stroke output increases during inspiration. This is due to the great increase in the stroke of the right ventricle, which is sufficiently great to mask the simultaneous diminution in left ventricular stroke volume. The mean arterial

[2] These prints were made directly from positive film, so the blacks and whites are naturally reversed.

blood pressures, when graphed on an appropriate scale, coincide at the majority of points with the changes in left ventricular stroke volumes (as would be expected) and are independent of the activity of the right ventricle. The effects of respiratory changes on ventricular volume and stroke were augmented by deep respiration and tracheal occlusion, diminished during quiet, shallow breathing, and eliminated during apnea induced by central superior laryngeal stimulation.

Differential blood pressures. To determine whether intrathoracic pressure changes are directly transmitted to the systemic arteries, contributing to the

INSPIRATION

	DIAS. VOL.	SYS. VOL.	STROKE VOL.
R.V.	555	460	95
L.V.	296	247	49
R.& L.V.	851	707	144

MEAN ARTERIAL BL. PR. 125 MM. HG.

EXPIRATION

	DIAS. VOL.	SYS. VOL.	STROKE VOL.
R.V.	489	468	21
L.V.	310	255	55
R.& L.V.	799	723	76

MEAN ARTERIAL BL. PR. 145 MM. HG.
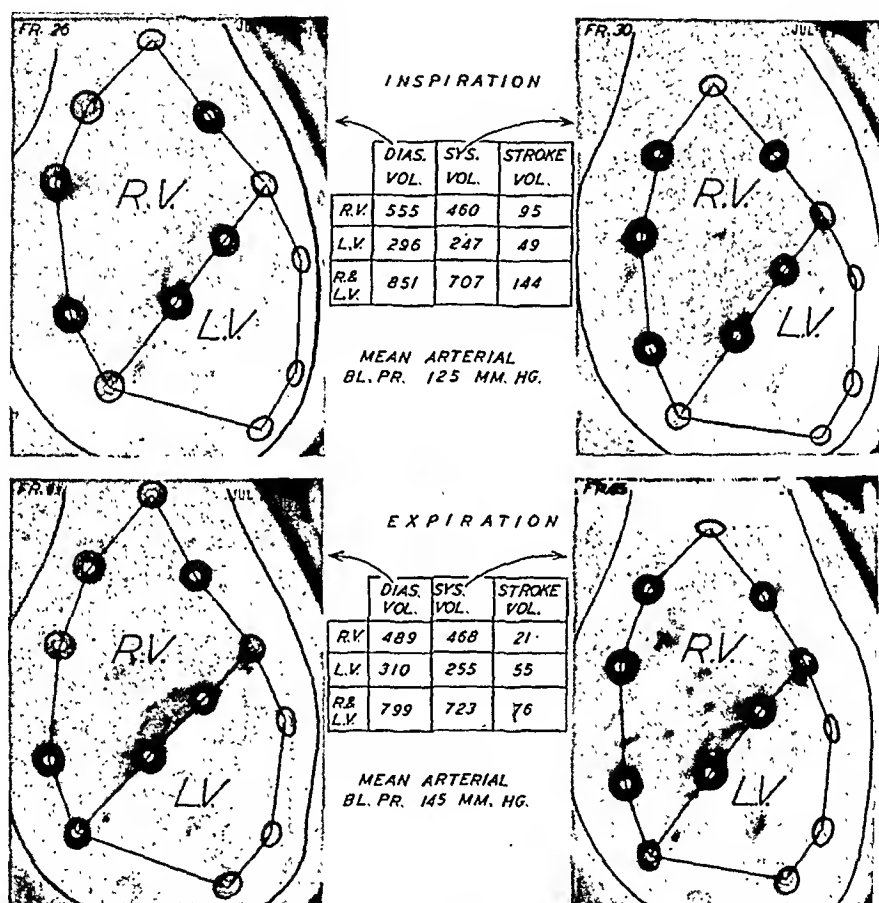
Fig. 5. Photographs of four selected movie frames, showing method of measuring areas of ventricles.

respiratory changes in arterial pressure, the following experiments were devised: Four dogs were trained daily to lie quiescent while femoral blood pressures were taken by direct arterial puncture. Simultaneous pneumograph and blood pressure recordings were taken on the Hamilton manometer. The animals showed definite sinus arrhythmia and a distinct drop in blood pressure which coincided with inspiration. The arrhythmias and respiratory variations were present even after the dogs had become accustomed to the procedure, at which time breathing was normal. Other records from animals which had been trained for

more than a year showed definite rhythmical variations in blood pressure, with or without sinus arrhythmias.

At the end of a two weeks' training period, the dogs were anesthetized with ether, and a cannula, stoppered airtight, was placed through the chest wall to communicate with the intrathoracic space. After a two day recovery period femoral pressure tracings were made with a differential manometer and the intrapleural cannula was connected to the tube leading to the outer·chamber of the differential manometer. Recording of intrathoracic pressure was done by means of a second tube attached to the intrapleural cannula. Controls taken before puncturing the artery showed a definite respiratory excursion of the differential membrane which corresponded to the simultaneous intrathoracic pressure tracing. Femoral pulse tracings were obtained while alternately clamping and opening the tube leading from the chest cannula to the manometer, so that ordinary and differential arterial pressures were obtained alternately.

If respiratory variations in peripheral pressures during normal breathing are caused to any appreciable extent by direct transmission of intrathoracic pressure changes, these respiratory variations should be diminished or eradicated by differential recording of intrathoracic pressure against blood pressure. Our data show that there is a slight elevation of the level of femoral pressures, and a reduction in the difference between the extremes of blood pressure at the peak of inspiration and expiration. There remain, however, variations in pressure with respiration which may be due in part to sinus arrhythmias and in part to changes in stroke volume, as described above. That changes in stroke volume are important are shown by the decrease in pulse pressure from expiration through inspiration.

DISCUSSION. Although the use of measurements of ventricular surface area changes is but a crude approximation of the volume changes within the ventricle itself, there seems to be little doubt that such measurements reflect the true changes in ventricular volume. The "volume" changes plotted in figure 3 seem to be true approximations to volume changes, because: 1, the contour of the plotted curves resemble oncometer volume curves, including an occasional typical diastasis, and even in some cases (not shown in fig. 3) there is a suggestion of the auricular "hump" which characterizes heart volume changes made with the oncometer; 2, there is a rather close correlation between the left ventricular "stroke volumes" and corresponding carotid pressures; 3, in accordance with the Starling principle, ventricular stroke volume bears a positive relationship to the diastolic size.

Strughold (1930) calibrated similar area measurements of the ventricles by injecting known volumes of fluid. He reported a geometrical relationship between volume and area within phsyiological limits. Burchell and Visscher (1941) criticise area measurements as a method of measuring heart volumes because of the angular displacement caused by rotation of the heart with each cardiac cycle. There is also the possibility that errors may be introduced by the rise and fall of the apex of the heart with inspiration and expiration, respectively. Since in our experiments the heart was cradled in the pericardium, rotation

was minimized, and mathematical constructions plus photographs of the heart with different degrees of inflation of the lungs show that the errors thus introduced are not only very small, but would tend to diminish, rather than increase, the effects of respiration upon stroke volume.

Apparently, then, there is an aspiration of blood into the right auricle and ventricle in inspiration. Through the operation of the Starling effect, the right ventricle increases its output. Despite this, the left ventricle fills less during this phase of respiration, presumably because of an increase in the capacity of the pulmonary bed, and the quantity of blood retained in the pulmonary vessels (see Burton-Opitz, 1921; Heinbeeker, 1927; Trimby and Nicholson, 1940; Cahoon, Michael and Johnson, 1941). This capacity increase is apparently sufficient to accommodate the greater amount of blood pumped by the right ventricle and, unless the inspiratory period is prolonged, to withhold it from the left heart until the onset of expiration. In some of these experiments, during the latter part of a long inspiratory pause, the left ventricular diastolic and stroke volumes tended to increase. It also seems probable that the elastic recoil of the pulmonary vessels during expiration tends to "force" blood out of the pulmonary vessels into the left heart, in addition to restricting the filling of the right heart. Thus, blood is sent to the lungs and retained there in larger amounts during the most advantageous time for aeration.

There is no doubt that, when it exists, a respiratory sinus arrhythmia also contributes somewhat to the blood pressure changes produced by the respiratory movements. In the absence of such an arrhythmia the carotid blood pressure fluctuations are due mainly to changes in the stroke volumes of the left ventricle.

SUMMARY

1. In dogs anesthetized by sodium barbital, direct cardiac volume changes were measured by means of an oncometer during normal breathing, with the ventricles exposed to intrathoracic pressure changes. During inspiration there was an increase in total diastolic size and stroke volume of the two ventricles. This confirms the findings of Boyd and Patras (1941).

2. Systemic arterial pressure decreased during inspiration in spite of increased total cardiac output during the same phase of respiration.

3. Under sodium barbital anesthesia, a portion of the ventral chest wall was removed, the heart exposed, and paper markers affixed to the heart so as to outline each ventricle. A window was sealed into the ventral chest opening, normal respiration was reinstated, and motion pictures were taken of the heart. Successive single frames were projected, the area of each ventricle measured in each frame, and these areas plotted together with simultaneous intrathoracic and carotid pressures. The right ventricle showed increased diastolic size and stroke volume during inspiration, while the same measurements on the left ventricle decreased; the reverse was true during expiration.

4. The increased diastolic size and stroke of the right ventricle during inspiration suggest that more blood is pumped into the lungs during that phase, while the decreased diastolic size and stroke of the left ventricle indicate that the blood is withheld from the left heart until the onset of expiration.

5. Systemic arterial blood pressure measurements on trained, unanesthetized dogs showed but slight reduction of respiratory influences on blood pressure when intrathoracic pressure influences were eliminated by the use of a differential manometer. These reductions are equivalent to simultaneous intrathoracic pressures, but are not sufficient to eliminate respiratory fluctuations in blood pressure.

6. The main factor responsible for the arterial blood pressure fluctuations of respiration is the changing output of the left ventricle. A sinus arrhythmia and direct influences of intrathoracic pressures may modify somewhat these blood pressure changes.

7. Each ventricle responds independently in accordance with the Starling principle, regardless of the diastolic size of the other ventricle.

REFERENCES

BOYD, T. E. AND M. C. PATRAS. This Journal 134: 74, 1941.
BURCHELL, H. B. AND M. B. VISSCHER. Am. Heart J. 22: 794, 1941.
BURTON-OPITZ, R. This Journal 7: 435, 1902.
        This Journal 58: 226, 1921.
CAHOON, D. H., I. E. MICHAEL AND V. JOHNSON. This Journal 133: 642, 1941.
EYSTER, J. A. E. AND E. V. HICKS. This Journal 104: 358, 1933.
HAMILTON, W. F., J. BREWER AND I. BROTMAN. This Journal 107: 427, 1934.
HEINBECKER, P. This Journal 81: 170, 1927.
HOOKER, D. R. This Journal 35: 73, 1914.
STRUGHOLD, H. This Journal 94: 641, 1930.
TRIMBY, R. H. AND A. C. NICHOLSON. This Journal 129: 289, 1940.
VISSCHER, M. B., A. RUPP AND F. H. SCOTT. This Journal 70: 586, 1924.
WIGGERS, C. J. AND L. N. KATZ. This Journal 58: 439, 1922.

THE ONE VENTRICLE PUMP AND THE PULMONARY ARTERIAL PRESSURE OF THE TURTLE: THE INFLUENCE OF ARTIFICIAL ACCELERATION OF THE HEART, CHANGES IN TEMPERATURE, HEMORRHAGE AND EPINEPHRINE

R. A. WOODBURY AND G. GORDON ROBERTSON[1]

From the Departments of Physiology and Pharmacology and Microscopic Anatomy, University of Georgia, School of Medicine, Augusta

A few instances are reported where humans with only one ventricle have lived to adult life (1). Most amphibia and reptiles have only one ventricle and yet these animals live in many environments over a large share of the globe and manage to reach maturity. Is the presence of only one ventricle in these animals an imperfect arrangement as it is often called (2, 3, 4) or does it provide certain advantages?

Previous studies have shown that turtles possess a very effective mechanism for increasing the systemic blood flow (5). In the present paper these studies are extended to include the pressure relationships, which are present in the pulmonary artery.

Inspection of the large vessels, which arise from the one ventricle of the turtle, shows dark red (venous) blood in the pulmonary artery, bright red arterial blood in the aorta supplying the head region and mixed blood in the aorta to the body. Greil (see 6) proved that very little mixing of the arterial and venous blood occurred in the ventricle. He injected a solution of sodium ferrocyanide into the pulmonary vein and after a systole observed the Berlin Blue reaction only in the blood from the aortas. He then injected the solution into the inferior vena cava and after a systole observed the Berlin Blue reaction only in the blood from the pulmonary artery.

The present conceptions of the mechanisms which influence the flow of blood through the turtle heart are based mainly upon the studies of Brücke and of Sabatier (6, 7, 8) and generally incorporate the following. Venous blood from the right auricle passes to the right side of the ventricle and arterial blood from the left auricle passes to the left side of the ventricle. The incomplete septum (interventricular ridge) and the spongy nature of the ventricular wall restrict mixing of the arterial and venous blood within the ventricle. During the contraction of the ventricle, the dorsal and ventral edges of the incomplete septum approach each other and according to some authors (2, 3, 8) separate the ventricle into two complete cavities. As a result the aorta to the head, which arises from the left side of the ventricle, receives mostly arterial blood. However, this does not explain why the pulmonary artery and the aorta to the body, which arise from the right part of the ventricle, receive venous and mixed blood respectively.

[1] Now with Louisiana State University School of Medicine, New Orleans.

According to Brücke (6, 7) the orifice of the pulmonary artery is closed towards the end of systole either by contraction of a ring shaped muscle bundle or by obstruction from the cartilaginous ridge which partly conceals this orifice.   Does this closure of the pulmonary orifice during the last part of systole contribute to the flow of arterial and venous blood into the proper vessels?

Pressure studies from the pulmonary artery and the aortas of turtles should supply crucial information concerning these mechanisms.

EXPERIMENTAL METHODS.   In six turtles the exposure of the blood vessels was made with the animal at room temperature (20–30°C).   This usually caused a rather large blood loss.   Six other turtles were kept in the refrigerator for several days.   Each of these was moved into the freezing compartment about thirty minutes before the experiment.   Exposure of the blood vessels was made with these animals in a pan filled with ice cubes.   The resulting blood loss was usually less than 1 cc.

Optical records of the blood pressure were obtained simultaneously from the pulmonary artery and from one or both of the aortas by means of the hypodermic manometer (9, 10).   One-half inch 26 G. needles were inserted into the vessels at equal distances from the heart.   Otherwise the technique was essentially as previously described (11, 5).

The pulmonary artery.   Serial cross sections (10 μ) of the pulmonary artery from its origin to a point beyond its bifurcation were prepared and alternately stained with 1, Delafield's hematoxylin and eosin; 2, Heidenhain iron hematoxylin, and 3, picrofuchsin.   The valvular attachment was found to extend peripherally from the base of the heart for a distance of 2.2 mm.   The cartilaginous ridge described by Brücke (6) located within the common wall of the pulmonary artery and the body aorta, extends peripherally from the base of the heart for a distance of 2.6 mm.   The pulmonary arterial wall in contact with the cartilage is composed entirely of fibrous connective tissue and endothelium.   In the remaining circumference cardiac muscle tissue is present.   It extends into the pulmonary artery 2 mm. beyond the base of the heart.   This cardiac muscle is mostly circular in arrangement but becomes oblique near the dorsal border of the. cartilaginous ridge.   Contraction of this cardiac muscle ring was observed sometimes during the last half or two-thirds of systole.   This narrows the first part of the pulmonary artery and together with the cartilaginous ridge, contributes to the obstruction of the pulmonary orifice (6).   At the level of the peripheral end of the cartilaginous ridge smooth muscle is present and progressively increases in amount so that from the point of bifurcation to the site of the arterial ligament smooth muscle forms a prominent portion of the arterial wall.

Beginning at the site of the arterial ligament (ductus Botalli or ductus arteriosus) each pulmonary artery is continued as a much smaller artery to the lung. This was first described by Brenner (see 12) but only one current reference (13) mentions it.

Longitudinal sections of the area were prepared.   Central to the site of narrowing many circular and longitudinal muscle fibers are present.   Data presented below (see effects of epinephrine) show that these muscles are capable of

activity and can influence the size and the elastic properties of the pulmonary arterial reservoir. Peripheral to the site of narrowing the vessel wall is thin with a corresponding decrease in the amount of fibrous, elastic and muscle tissue.

Pressure relationships among pulmonary and systemic vessels. Pressure pulses from the right and left aortas are synchronous and show that the pressures in these two vessels are equal (fig. 1). This suggests that contrary to accepted conceptions (2, 3, 8) the interventricular ridge does not form a complete barrier during systole between the left and right sides of the ventricle.
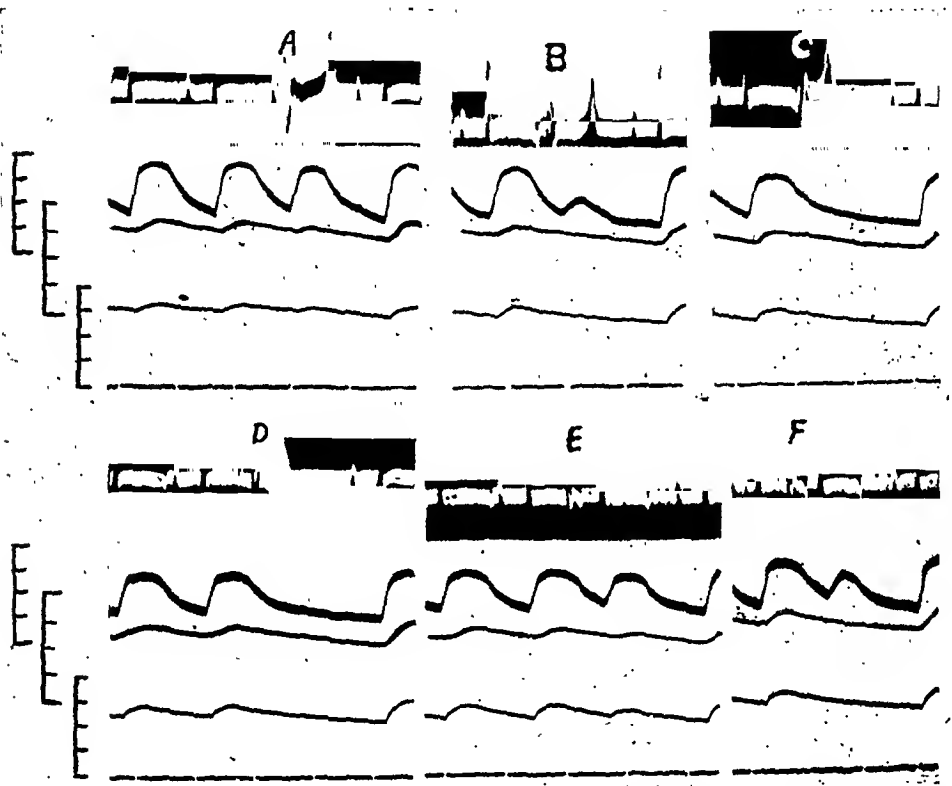
Fig. 1. Simultaneous electrocardiograms (lead 2) and pressure pulses from the pulmonary artery, right aorta and left aorta (from above downwards). The letters designate the electrical stimuli which were applied progressively earlier in the cardiac cycle. Stimuli A, B, C and D cause ventricular complexes; E and F cause auriculo-ventricular contractions. In these and all subsequent records the blood pressure scales are shown in units of 10 mm. Hg and the base line is interrupted at intervals of 1 second.

The pulmonary pressure pulses, however, differ in four main ways from those of the aortas (fig. 1, 2). First, the pulmonary contours show a rapid descent of pressure during diastole. This means that, like mammals, the pulmonary peripheral resistance is small in relation to the systolic change in the volume of the pulmonary arterial reservoir ("Windkessel"). Second, as in mammals, the diastolic pressure in the pulmonary artery is much less than that in the systemic arteries. Third, the abrupt systolic increase in the pulmonary pressure always precedes the systolic rise in the aortic pressure by the length of time required to

raise the pulmonary pressure to the level of the aortic diastolic pressure. Since venous blood occupies the right part of the ventricle (6), this initial blood flow into the pulmonary artery is the venous blood. Fourth, during the last half or two-thirds of systole, the pulmonary pressure is 2 mm. Hg or more below that in the aortas (fig. 1, 2). This difference of pressure can be explained only by some interference with blood flow into the pulmonary artery. The obstruction produced by the cartilage (6) accounts for small differences in pressure. Contraction of the cardiac muscle ring was observed only when the records showed that large differences in pressure were present.

Effects of artificial excitation. As electrical stimuli are applied progressively earlier in diastole the pressure pulses of the premature contractions progressively decrease in size (fig. 1-A, B, C, D). Stimuli very early in diastole (during the T wave) cause ventricular contractions which pump little if any blood into any of the vessels (fig. 1-C, D). When the stimuli were applied during the first part of the T wave, delayed contractions (14) were often present (fig. 1-E). Such contractions produced definite aortic and pulmonary pressure pulses, but electrocardiograms showed them to be auricular ventricular contractions. The contracting auricles pump blood into the relaxing ventricle. Applying the stimulus still earlier (fig. 1-F), the premature auricular ventricular contraction pumps only sufficient blood from the auricles through the ventricle to cause a pulmonary pulse but no aortic pulse.

The data fail to support the statements that the ventricle of the turtle retains a significant volume of blood at the end of systole and that the presence of such residual blood provides the mechanism by which minute output can be increased (14). The ventricle of the turtle retains only an insignificant amount of blood at the end of systole.

Artificial acceleration of the heart by means of repeated electrical stimuli lowered the mean systemic arterial pressure (14, 5). Pulmonary pressure pulses, however, showed that the mean pulmonary pressure was increased. This is explained by a shunting of some of the cardiac output into the pulmonary circulation. As shown in figure 1 A, premature contractions pump less than the usual amount into the aortas, but may pump almost the usual quantity into the pulmonary artery. Repeated electrical stimuli, by accelerating the heart and by shifting the distribution of the blood, increased the mean pulmonary and lowered the mean systemic pressures. Such artificial acceleration, however, produces effects which differ from those where the increase in heart rate is governed by body temperature and is coördinated with increased venous return and metabolic needs (5).

Influence of the body temperature. Cooling the turtle slows the heart, decreases aortic pressure, and reduces systemic blood flow (5). It also lowers the pulmonary systolic and diastolic pressures (fig. 2). In three turtles the pulmonary pressure averaged 40/15 mm. Hg when the body temperature was approximately 35°C; 27/9 mm. Hg at 20°C; and 20/4 mm. Hg at 5°C.

Cooling the animal increased slightly the slope of the pulmonary diastolic pressure die-away curve at any given pressure. This might be secondary to the

smaller pulmonary venous pressure (see below), which, of course would facilitate blood flow from the pulmonary artery. The absence of any decrease in the slope indicates that vasoconstriction of the peripheral pulmonary vessels was ineffective if present. Since cooling the turtle does cause effective vasoconstriction of the systemic vessels (5), the decreased cardiac output associated with the slow heart rate tends to reduce systemic flow more than pulmonary flow.

The turtle, however, can avert any extreme shifting of blood flow. As the heart slows and the pressures decrease, the pulmonary curve no longer is steep during the last part of diastole, though the aortic contours continue to show a gradual descent of pressure throughout diastole (fig. 2). Such records show that at these low body temperatures blood flow from the pulmonary artery occurs principally during systole and early in diastole and is quite small during the last part of diastole. On the other hand, systemic flow though decreased by vasoconstriction continues throughout diastole.
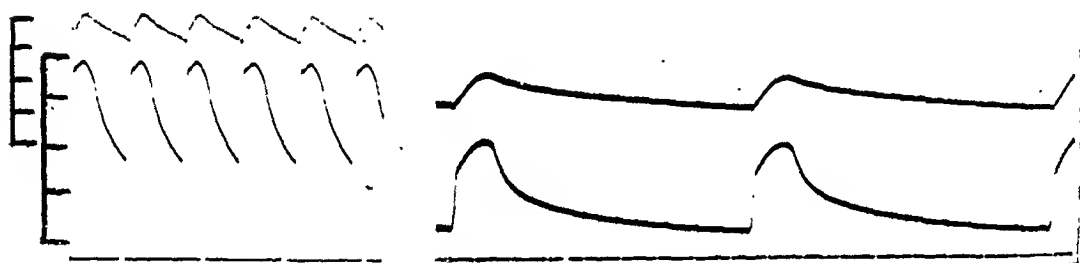
Fig. 2. Pressure pulses from the aorta and the pulmonary artery. The record at the left is from a turtle, while the body temperature was approximately 35°C; that at the right is from another turtle while the body temperature was approximately 2°C.

As the body temperature approaches 0°C and the heart rate becomes extremely slow (2 to 4 beats per minute), systole, though long, occupies as little as 10 per cent of the cardiac cycle. Pulmonary outflow practically ceases during the greater part of the unusually long diastoles, but systemic outflow continues throughout these long diastoles. These data indicate the presence of an economical means of maintaining adequate distribution of blood between the pulmonary and systemic circulations at these low temperatures.

During these long diastoles the color of the blood in the ventricle changes from that of arterial to that of venous blood. Evidently the main blood flow into the ventricle early in diastole is oxygenated blood from the pulmonary vein. This would explain the color of the ventricle early in diastole and may cause an unusually low pulmonary venous pressure. The blood flow into the ventricle from the systemic veins is probably slower and probably continues throughout diastole.

This extreme slowing of the heart is of vagal origin for it can be eliminated either by administering atropine sulfate (2 mgm.) or by pithing the animal.

Effects of hemorrhage and infusion of fluid. The blood pressure changes, which

were caused by moderately severe hemorrhage, are shown by the reconstructed pressure pulses, which are presented in figure 3. After hemorrhage the aortic and the pulmonary blood pressures are unusually low considering the body temperature of the animal. The aortic pressure remains elevated throughout systole, but the pulmonary pressure curve differs from the normal in that the pressure declines rapidly during the last half of systole.

The intravascular injection of 8 to 10 cc. of Ringer's solution raised the pressures and caused the pulse contour to return to normal (see first and second records of fig. 4). Severe hemorrhage was then produced and the pressures and pulse contours again showed the effects of hemorrhage (see second and third records of fig. 4).

Direct observation showed that after these hemorrhages the muscular tissue at the orifice of the pulmonary artery was contracting during the last part of systole. It did not occur after infusion of fluid. This shows that the pulmo-
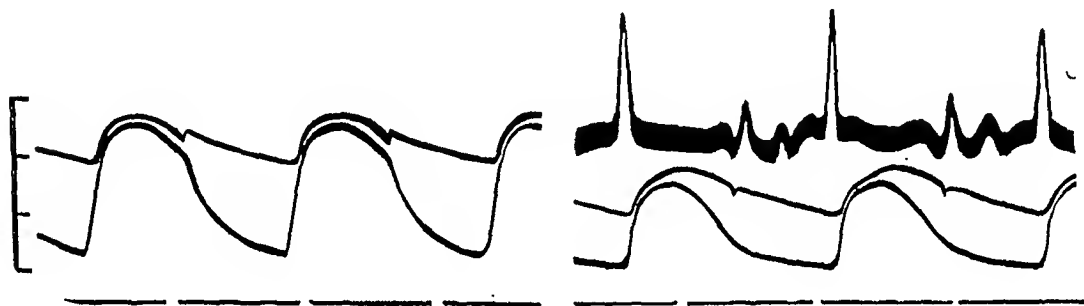
Fig. 3. Aortic and pulmonary arterial pressure pulses reconstructed to the same scale. The aortic contours were lifted 2 mm. Hg to avoid superimposition. Records are from a cooled turtle where the blood loss during the exposure was less than 1 cc. After taking the records at the left, moderate hemorrhage was allowed to occur and the records at the right were then obtained. An electrocardiogram recorded simultaneously is included with the right records so that the duration of systole is clear.

nary muscular ring at the orifice becomes active and plays an important rôle when the blood volume is abnormally low (hemorrhage and anhydremia). By contracting during the latter part of systole, it shuts off the flow of blood to the pulmonary artery and allows the ventricle to pump blood into the two aortas.

This is a rather ingenious and effective reserve mechanism for maintaining aortic pressure levels.

After severe hemorrhage in a turtle whose pulmonary arteries were severed near the arterial ligament, a definite sustained contraction ring appeared immediately central to the site where the pulmonary artery divides into the right and left branches. Microscopic examination showed the presence of a large amount of muscle tissue as described above and in some turtles a cartilaginous structure somewhat similar to that at the orifice of the pulmonary artery.

In a turtle, where hemorrhage was insignificant, Ringer's solution was injected rapidly into the left aorta throughout one diastole. The pressure in this vessel did not decrease during this diastole, but increased 5 mm. Hg. The pres-

sure in the other aorta did not decrease during this diastole, but increased 2 mm. Hg.   The pulmonary pressure, of course, was not influenced.   These data prove that anastomoses are present between the two aortas.

These anastomoses (through the dorsal aorta (3)) may be unimportant in the normal physiology of the turtle, but their presence disproves statements such as "the 'imperfect' ventricle cannot be remedied merely by completing the interventricular septum" (2).   The aorta from the right ventricle receives mixed arterial and venous blood.   Merely completing the septum would allow only venous blood a direct entrance to this aorta.   The right ventricular output would be decreased and its systolic pressure would be lowered.   The left ventricular output would be increased, since all of the arterial blood would pass directly into its aorta.   The systolic pressure on the left side of the heart and in
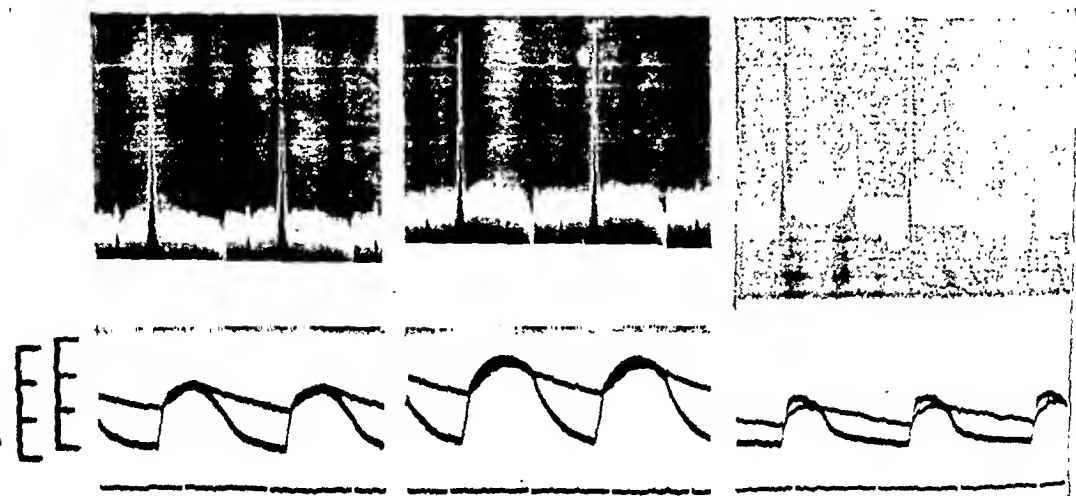
Fig. 4. Electrocardiogram and pressure pulses from the left aorta and pulmonary artery. Left pressure scale = aorta; right pressure scale = pulmonary artery.   Records are from a cooled turtle where moderate hemorrhage occurred during exposure of the vessels.   Approximately 10 cc. of Ringer's was injected into the left aorta during the first break in the record.   At the second break in the record severe hemorrhage was allowed to occur.

its aorta would be increased.   The resulting differential pressure between the two aortas would supply ample arterial blood through the anastomoses.   Active blood flow through these anastomoses would soon bring about their enlargement. The end result might be similar to that found in the crocodile and alligator, both of which have completed their interventricular septum.   However, the mere completion of the septum would deprive the turtle of the advantages of a heart with only one ventricle.   This might restrict his natural habitat to that of the crocodile and alligators, i.e. to areas between the frost lines of the northern and southern hemispheres.

Effect of epinephrine HCl.   The intravascular injection of 0.2 mgm. of epinephrine HCl (fig. 5) increased the systolic pressure equally in all three vessels, but increased the diastolic pressure only in the aortas.

A study of the pulse contours shows that epinephrine produces constriction of the peripheral systemic vessels and constriction of the large vessels which form the pulmonary arterial reservoir ("Windkessel"). It has been shown in mammals (11) that peripheral vasoconstriction of systemic vessels raises the pressure without producing any fundamental change in the type of curve of the diastolic portion of the pressure pulse. The diastolic portion of the pulmonary pressure pulse, however, does show a fundamental change in the type of curve after the injection of epinephrine (fig. 5). At equal pressures during the lower part of the curve the steepness was definitely increased by epinephrine, while at equal pressures at the upper part of the curve epinephrine produced no change or even some decrease in the steepness of the curve. Such changes are not produced by a change in peripheral resistance (11), but are produced by changes in the elastic properties of the "Windkessel" from constriction of the large arteries (15).
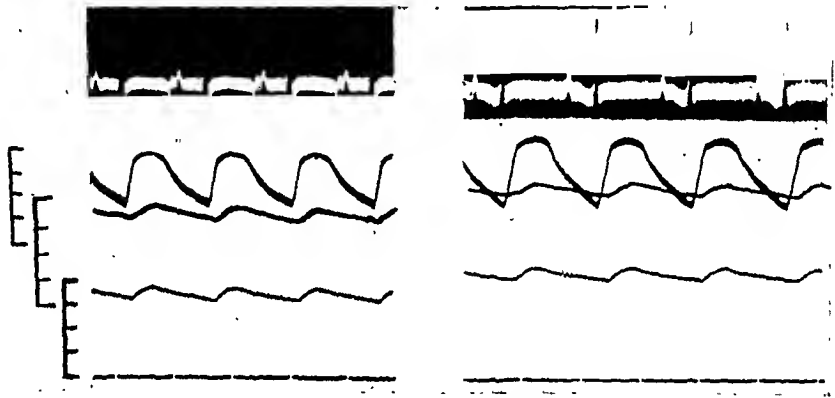
Fig. 5. Pressure pulses from pulmonary artery, right aorta and left aorta (from above downwards). At break in the record 0.2 mgm. of epinephrine HCl was injected into the left aorta and twenty seconds of records were deleted.

Therefore, in turtles, the injection of epinephrine produces constriction of peripheral systemic vessels and of the vessels forming the pulmonary reservoir. No evidence was obtained that epinephrine effectively constricts the peripheral pulmonary vessels.

SUMMARY AND CONCLUSIONS

The right and left aortic pressure pulses of turtles are synchronous and show equal pressures. Blood flow and the systolic pressure rise occurs slightly earlier in the pulmonary artery than in the aortas. During the last part of systole the pulmonary pressure becomes 2 or more mm. Hg below that in the aortas. During diastole the pulmonary pressure descends more rapidly and to a lower value than that in the aortas.

No evidence was obtained that the ventricle retains any significant residual

volume of blood at the end of the ejection period.   The cardiac output in turtles is increased by an increased heart rate and/or an increased diastolic filling of the ventricle.

Cooling or warming the turtle respectively lowers or elevates the pulmonary systolic and diastolic pressure.   At body temperatures near 0°C, diastole is excessively prolonged.   This is vagal in origin.

The presence of only one ventricle enables the turtle to regulate effectively the distribution of blood flow between the systemic and pulmonary areas.   If the need arises (after hemorrhage) blood flow can be diverted into the systemic vessels by closing off the orifice of the pulmonary artery during the greater part of systole and by reducing the size of the pulmonary arterial reservoir.

Epinephrine HCl administered intravascularly increased the peripheral resistance of the systemic vessels, increased the muscle tone of the great pulmonary arteries, but gave no evidence of any effect upon the peripheral resistance of the pulmonary circulation.

REFERENCES

(1) YOUNG.   J. Anat. and Physiol. 41: 190, 1906–07.
(2) HYMEN.   Laboratory manual for comparative vertebrate anatomy.   Univ. of Chicago Press, Chicago, 1922.
(3) PARKER AND HASWELL.   Textbook of zoology.   Volume 2, Macmillan and Co., London, 1897.
(4) POHLMAN.   Anat. Record 3: 75, 1909.
(5) WOODBURY.   This Journal 132: 725, 1941.
(6) WINTERSTEIN.   Handbuch der vergleichenden physiologie.   Band 1, Verlag von Gustav Fischer, Jena, 1925.
(7) BRÜCKE.   Denkschr. d. k. Akad.   Wis. math.-wiss. Cl., Bd. 3, Wien, 1852.
(8) SABATIER.   Etudes sur le coeur dans la serie des vertebres.   Montpellier, 1873.
(9) HAMILTON, BREWER AND BROTMAN.   This Journal 107: 427, 1934.
(10) HAMILTON, WOODBURY AND HARPER.   J. A. M. A. 107: 853, 1936.
(11) WOODBURY AND HAMILTON.   This Journal 119: 663, 1937.
(12) O'DONOGHUE.   J. Anat. and Physiol. 51: 137, 1916–17.
(13) NOBLE AND NOBLE.   Brief anatomy of the turtle.   Stanford University Press, Stanford, Calif., 1940.
(14) SHANNON AND WIGGERS.   This Journal 128: 709, 1940.
(15) McWILLIAM.   Proc. Royal Soc. 70: 109, 1902.

FROZEN PLANT JUICE AS THE SOURCE OF
A RABBIT OVULATING FACTOR[1]

R. BORASKY AND J. T. BRADBURY

From the Division of Nutrition and Physiology, Bureau of Dairy Industry, United States Department of Agriculture

Investigations of the ovulation inducing factor obtained from plant sources have been retarded by the lack of a year round supply of material. Dry leaves and even the fresh juice of the oat plant have not been a consistent source of the activity (1, 2). Extracts containing this factor have been prepared from occasional samples of frozen oat juice obtained from a commercial laboratory, but the lack of tests for activity of the juice before it was frozen and the frequent failures to obtain potent extracts from such juices, raised doubts as to the retention of the activity during frozen storage.

During the past year portions of juice samples, obtained at various times during the growing season, were canned and stored in the freezing room. The juices so stored were tested in the estrous rabbit from time to time to determine whether those samples, which were active as fresh juice, would retain their potency and be a source of material for study during the winter months when fresh juice would not be available. The data obtained during this investigation show that frozen juice retains its potency, and also that there is a seasonal variation in the response of the rabbit to this plant substance.

MATERIALS AND METHODS. Each collection of oat plants was brought in from the field and immediately ground to a pulp in a power grinder and then the juice was expressed from the pulp by squeezing through unbleached muslin. The major portion of the juice was sealed into tinned cans, each containing about 1 liter, which were then put in the freezing room for storage at 0–5° F. An aliquot of the remaining juice was immediately clarified by centrifugation or filtration, and the clarified juice processed in the same manner as described by Friedman and Mitchell (2). The benzoic acid powders obtained by this method were extracted with water at pH 7.4 (pH adjusted with 0.1 N NaOH) and the extracts were assayed by the rabbit ovulation test. The stored juices were processed and assayed after periods varying from a few days to several months. Since March 1942, the activity was extracted by a simplified procedure. The hydrogen ion concentration of the thawed juice is adjusted to 7.4 with 3 N NaOH, and allowed to stand in the refrigerator at 4°C. over night. The juice is then clarified by centrifugation, the solids are discarded, and the clarified juice then adjusted to pH 4.0 with 6 N H_2SO_4. This causes the formation of a finely divided precipitate which settles out more completely when placed in the refrigerator for several hours. The clear supernatant fluid is siphoned off and the

sediment is centrifuged. The supernatant liquor is discarded; and the wet precipitate, which contains the activity, may be extracted with water (about $\frac{1}{10}$ to $\frac{1}{20}$ of the original volume) at pH 7.4 and this extract injected intravenously for assay, or the precipitate may be washed with acetone, dried under reduced pressure, and stored as a powder for future reference.

The simplified method outlined above, is based on the observation that the addition of benzoic acid to plant juices always produced a final pH of 4.0 ± 0.1. The aqueous extracts of the precipitates, thrown down at pH 4, are less toxic than those from the benzoic acid precipitation, and there is a high percentage of recovery of the active principle (cf. sample 06 below).

RESULTS AND DISCUSSION. The results of repeated tests on 3 different lots of frozen juice are plotted in figure 1 as representative of 6 different collections of oats. Each symbol represents an aliquot portion of a particular sample that
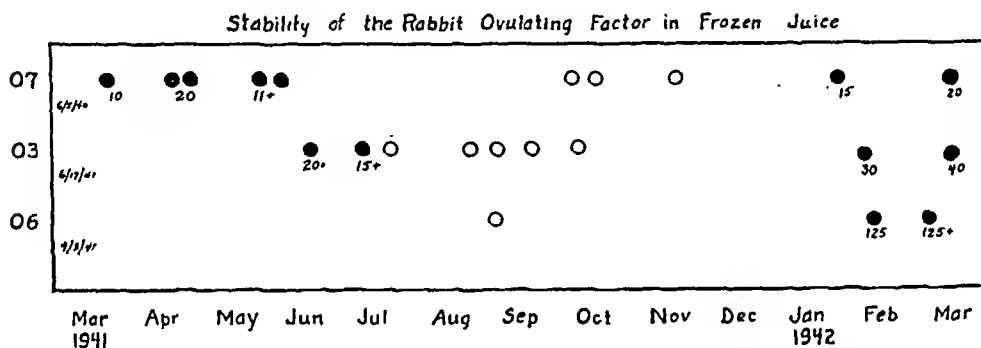
Fig. 1. A series of assays of 3 different lots of oat juice extracts in the estrous rabbit. The solid circles represent positive tests (induced ovulation) and the figure below them indicates the number of ovulating units per liter of juice. The open circles represent negative tests. The date of collection for each juice is noted. The first tests on 03 and 06 were on the fresh juice extracts. We are indebted to Dr. E. T. Gomez and Mr. A. M. Hartman of this Bureau for the samples of the juice, designated 07, which were collected and put in storage June 5, 1940.

was processed and assayed at the time indicated. It is apparent that juices, when frozen, retain their rabbit ovulating potency for at least 21 months. The apparent increase in potency of the juices in the March 1942 assays may be due in part to the improved method of extraction. Both juices, 07 and 03, are peculiar in that samples which were assayed in rabbits from August through November, gave negative results. Juice 06, collected September 3, 1941, was negative when tested initially; but, after 6 months of storage, it proved to be the most potent juice obtained last year. It is interesting to note that 8 to 10 cc. of the clarified juice (06) when injected intravenously, after adjusting to pH 7.4 with 0.1 N. NaOH, has induced ovulation in 3 different assays. Doses of extracts representing 10 cc. of this juice and containing 1.5 mgm. of dissolved solids, have also been effective in inducing ovulation in the rabbit. This indicates a high percentage of the original activity in the final extracts. Another sample of oats collected September 9, 1941, was similar to sample 06 in that it also was

negative when assayed initially but has subsequently been proven to be of similar potency.

Friedman and Mitchell state that when extracts prepared during the preceding season had been stored as powders that, "With such stored powders it was possible to obtain positive responses in every month from January to May inclusive, . . ." In our experience, extracts from frozen juices have induced ovulation in the months of January into July. It was possible to obtain positive responses in rabbits from March to July 1941, and again from January to March 1942, to doses of juice extracts which gave negative responses from August to December 1941. During the late summer and fall there is a lower incidence of estrus in rabbits (3). Our observations on extracts from frozen juice and the reconsideration of the data of Friedman and Mitchell (4) make it seem very probable that there is a seasonal variation in the response of the rabbit to this plant substance.

SUMMARY

Oat juice collected at various intervals during the growing season retains its ovulating potency for at least 21 months when stored in the frozen state. Such storage makes possible a reliable year round source of the ovulation factor in plant juices. A simplified method for the extraction of this factor is presented. There is a variation in the responsiveness of the estrous rabbit to the ovulation factor, the rabbit being less responsive during the late summer and fall months of the year.

REFERENCES

(1) FRIEDMAN, M. H. AND G. S. FRIEDMAN. This Journal 125: 486, 1939.
(2) FRIEDMAN, M. H. AND J. W. MITCHELL. Endocrinology 29: 172, 1941.
(3) FRIEDMAN, M. H. Ann. Rev. Physiol. 3: 620, 1941.
(4) MITCHELL, J. W., R. BORASKY AND J. T. BRADBURY. Endocrinology 31: 283, 1942.
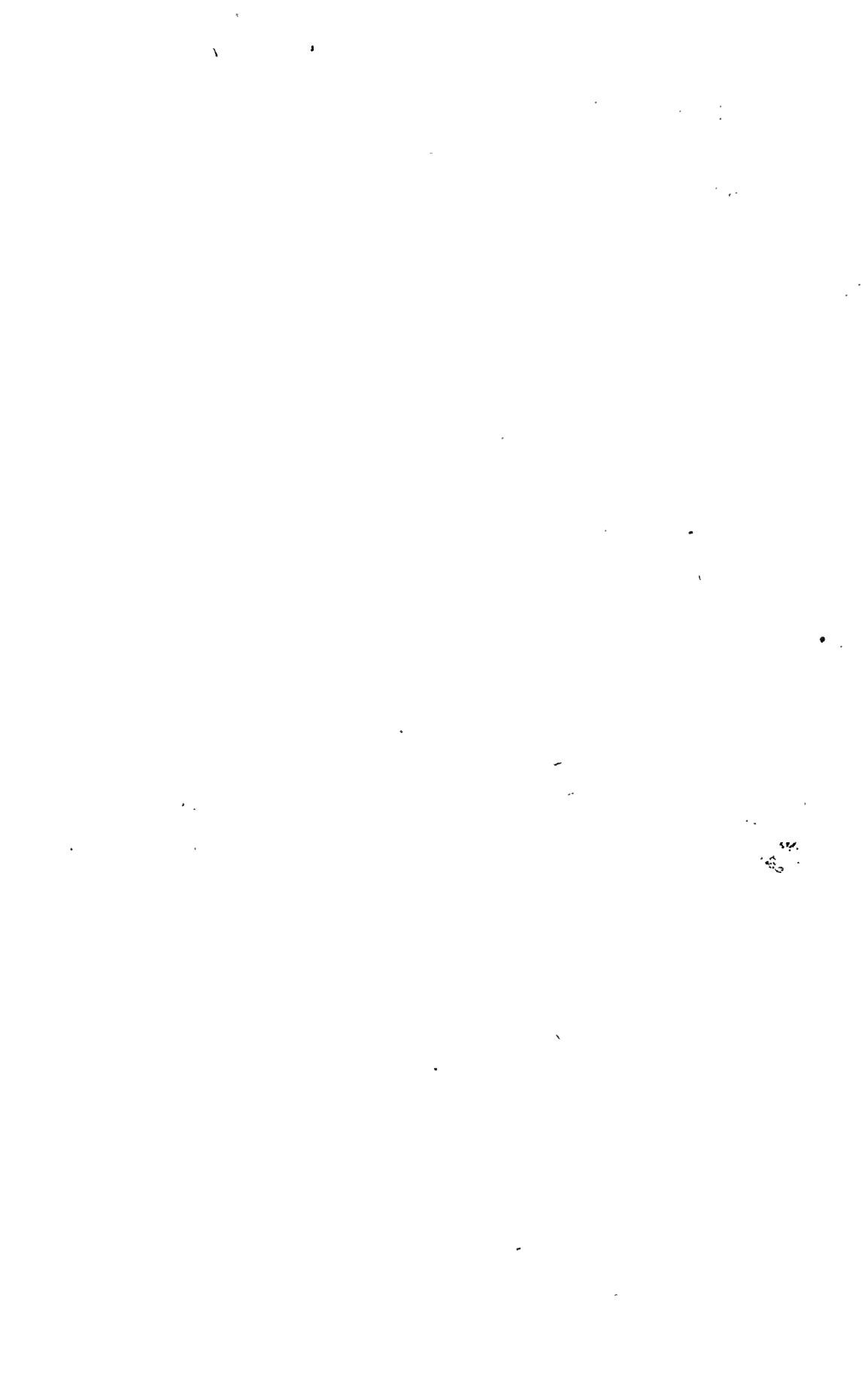
THE EFFECTS OF HEIGHTENED NEGATIVE PRESSURE IN THE CHEST, TOGETHER WITH FURTHER EXPERIMENTS UPON ANOXIA IN INCREASING THE FLOW OF LUNG LYMPH

MADELEINE F. WARREN, DELORES K. PETERSON AND CECIL K. DRINKER

From the Department of Physiology, Harvard School of Public Health, Boston, Massachusetts

In 1921 Graham suggested and showed experimentally that abnormally high negative pressure in the chest might cause pleural transudates, particularly if pulmonary edema was already present, fluid being sucked from the lung capillaries into the pleural sacs. Brock and Blair (1931), utilizing a heart-lung preparation, actually saw bloody fluid exude from the surface of the lungs under experimental conditions which permitted increased negative pressure within the glass chamber containing the lungs. Yamada (1933) made pleural punctures in several hundred Japanese soldiers. When the puncture followed a period of rest he obtained small amounts of fluid in 29 per cent of his subjects. If, however, the same individuals exercised vigorously, thus going through a period of violent breathing, Yamada obtained fluid on puncture in 70 per cent of his subjects. He did not require the men to breathe against resistance, that is, to inspire through a tube containing cotton wool or in some way arranged to make inspiration difficult. That situation, coupled with work sufficient to produce *deep* breathing or with inhalation of 5 to 10 per cent carbon dioxide mixed with air or oxygen to accomplish the same purpose, would in all probability have produced even more marked results than those gained through exercise alone, since maximum degrees of negative intrathoracic pressure may be produced by such conditions.

In the first group of experiments which follows, the flow of lymph from the lungs and heart has been observed during breathing against abnormal inspiratory resistance.

EXPERIMENTS AND DISCUSSION. *Anatomical considerations.* In a previous paper Warren and Drinker (1942) described the collection of lymph from the lungs of dogs. They showed that lymph entered the right lymphatic duct from all parts of the lungs except the upper section of the left lung, and that this lymph in no case contributed materially to the lymph flow from the thoracic duct. This was a surprising finding to the authors, though to a degree described by anatomists for man (Sappey, 1874; Rouvière, 1932).

The facts of the situation for the dog are shown in figure 1.   In the left semi-diagrammatic drawing, the heart, great vessels, and inflated lungs are shown as they would appear if the anterior wall of the chest were removed.   So far as the lymphatics from the lungs are concerned, the superior vena cava is an important guide.   If it is followed upward in the illustration, the right lymphatic duct is seen entering the right subclavian vein just above a fairly constant lymph node. Between the superior vena cava and the aorta, two or more nodes are found which lie upon the surface of the trachea.   The lymphatics entering this chain carry lymph from the heart and lungs.   In the drawing, the vessels to and from
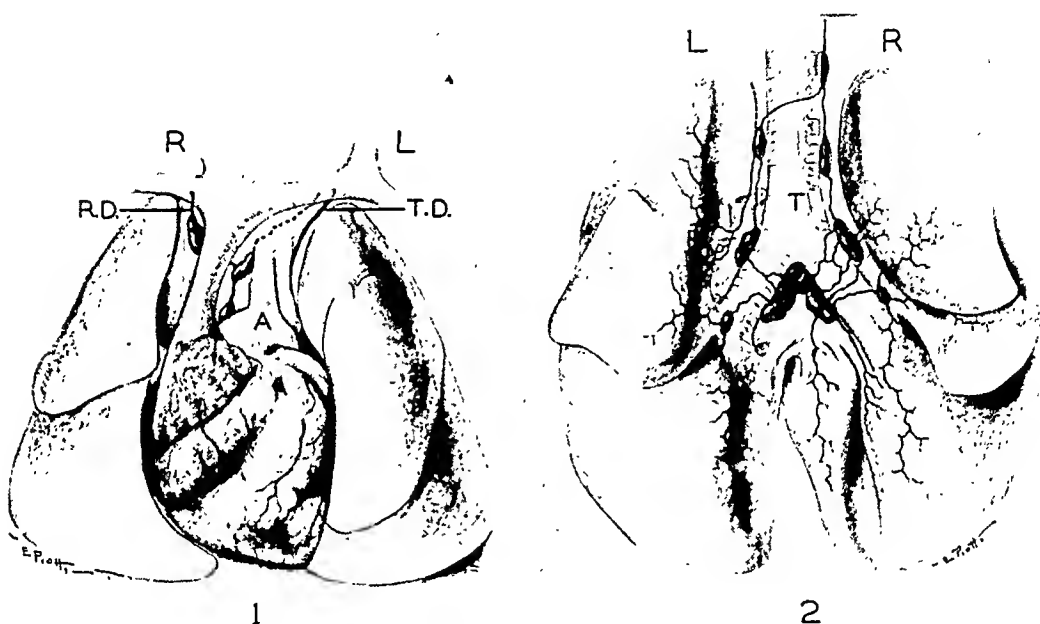
Fig. 1. The diagrammatic drawing (1) to the left shows the heart, lungs and great vessels of the dog viewed anteriorly.   The superior vena cava is the prominent landmark.   On the right, there is a single node not far below the subclavian vein.   To the left of this vessel two other nodes are usually found, and from the upper an inconstant vessel may occasionally cross to the thoracic duct.   The drawing (2) to the right illustrates the fact found in almost all cases that lung lymph drains ultimately to the right side.   It is a rear view of the lymphatic system and lungs of the dog.

the nodes are single.   As a matter of fact a number of afferent lymphatics enter each node, and with experience one can learn to distinguish the smaller cardiac lymphatic going to the upper node between the superior vena cava and the aorta, from several other afferent vessels carrying lymph from the lungs.

The right drawing (2, fig. 1) shows the gross features of lymph drainage from the lungs of the dog viewed from the rear.   Drainage of lymph to the right is clearly evident.   In both 1 and 2, figure 1, a vessel is shown as a broken line. Such vessels represent infrequent connections, in 1 crossing from the right to the thoracic duct or to enter the subclavian vein independently, and in 2 ascending directly to join the thoracic duct.   Neither of these connections is large, and if

present they are unimportant since the lymph diverted through them from the right duct cannula is insignificant in amount.

Many dissections have shown two things. First, in about three out of every six dogs the right lymphatic duct contains chyle. Sometimes the duct may be as large or larger than the thoracic duct, and is the main avenue for delivery of fat from the intestine to the circulation. Happily this irregular state of affairs is readily detected if right duct and thoracic duct lymph are compared, and it is found that the former is a clear fluid with 2 to 3.5 per cent protein, few red cells, and varying numbers of lymphocytes, while in the latter there is the milky opacity, which the chyle provides, together with a considerable number of red cells and a higher percentage of protein. Obviously, when the right duct lymph contained chyle an experiment upon lung lymph could not be done unless the abdomen was opened and the thoracic duct ligated just below the diaphragm. This has not been done in the experiments which follow.

These facts make it possible to collect lymph which in flow and composition represents that coming from the lungs, and in such a preparation one obtains practically all the lung lymph plus lymph from the heart which, in the absence of anoxia, will not, however, change unless cardiac activity is increased or decreased markedly, contingencies against which it is possible to provide adequate control. Cannulation of the right lymphatic duct has been a difficult task and is accomplished satisfactorily in about one out of five animals. If one wishes lung lymph alone, there is as yet no way of collecting it short of opening the anterior mediastinum under artificial respiration and cannulating a lung lymphatic, the procedure followed in the experiments upon anoxia reported in the latter part of this paper.

Experiments in which intrathoracic negative pressure was increased. A dog was anesthetized with sodium barbital given intravenously. This barbiturate is preferable to nembutal when respiration must be kept constant. Under nembutal the animal tends steadily to emerge from anesthesia, and this is particularly disturbing in respiratory experiments since new injections of the anesthetic are invariably depressing for a short period. The right lymphatic duct was cannulated and produced clear chyle-free lymph. At the start, the animal breathed 100 per cent oxygen under conditions precluding any possibility of resistance to inspiration. It was possible, however, by turning a three-way valve to compel the animal to breathe pure oxygen through a tube containing cotton wool. The obstruction to inspiration was very great, the negative pressure in the chest measured by means of a needle thrust through the chest wall reaching 56 mm. Hg at the height of inspiration. Clearly the experiment imposed a very severe strain which the animal could not have endured for many minutes. There was an immediate increase in lymph flow, and the lymph at once began to show red cells. Prior to the onset of obstructed inspiration the lymph contained 50 red cells per cubic millimeter. In 7 minutes the red cell content of the lymph rose to 57,900 per cubic millimeter. During this period of difficult inspiration the systemic blood pressure fell from a very even level of 143 mm. Hg. to 112 mm. Hg.

It was thus apparent that a large increase in negative intrathoracic pressure

drew fluid from the lung capillaries into the lung parenchyma and, under the excessive strain imposed by this experiment, not only plasma but red cells left the lung capillaries to be returned to the circulation in the lung lymph. When the obstruction to inspiration was removed the red cell content of the lymph disappeared at once, an interesting commentary upon the rapidity with which non-
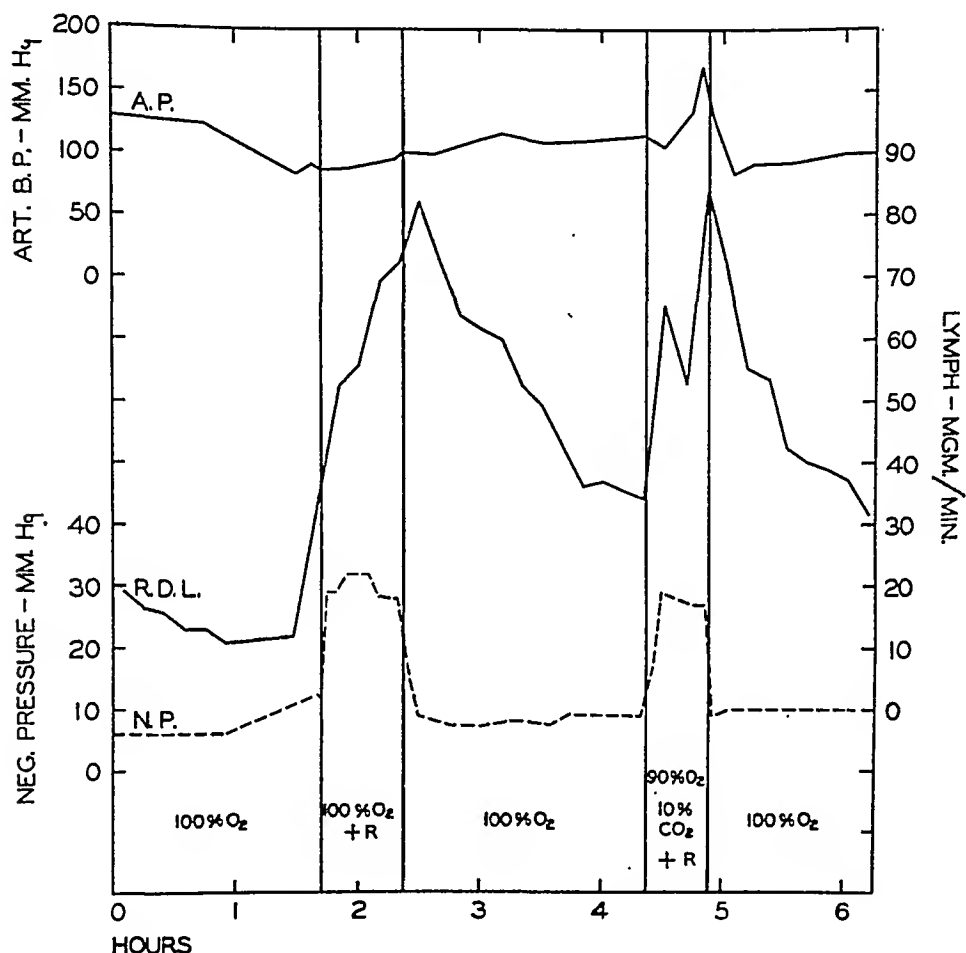
Fig. 2. The effect of breathing against inspiratory resistance. *Upper curve, A.P.,* arterial blood pressure; *middle curve, R.D.L.,* lymph flow in milligrams per minute; *lower curve (broken line), N.P.,* negative pressure in chest. During the first hour and a half lymph flow fell from nearly 20 to 11 mgm. per minute. Resistance to inspiration was then suddenly increased, and lymph flow rose at once to 82.5 mgm. per minute. When resistance was removed lymph flow fell to 34.0 mgm. per minute, but again increased sharply when the dog breathed 90 per cent oxygen and 10 per cent carbon dioxide with resistance.

ameboid cells reach avenues of lymphatic drainage in the moving, breathing lung.

Figure 2 summarizes the results of a more extended experiment. In this case a dog anesthetized with sodium barbital was prepared as follows. The trachea was cannulated in order to permit inhalation of oxygen or oxygen plus carbon

dioxide; the external jugular vein on the right side was made ready for introduction of a long glass tube passed down through the superior vena cava into the right auricle in order to obtain samples of mixed venous blood; blood pressure was taken from one femoral artery, and arterial blood samples under oil from the other. The right lymphatic duct was cannulated and found to produce chyle-free lymph.

There is little need to comment on the results as shown in figure 2. It is clear that increased negative pressure in the chest produces a huge increase in lymph flow. It may be contended that with the added respiratory effort there would be larger inflow into the heart and so more possibility of lymph production in the lungs. Cardiac output, determined by utilizing the direct Fick principle (Grollman, 1932), was 1.34 liters per minute prior to the first period of resistance to breathing, and at the height of the first period of resistance to inspiration rose to 1.42 liters per minute.

In the first period of resistance to inspiration, which is shown so dramatically in figure 2, certain other points deserve comment. First of all, during the entire experiment the oxygen content of the arterial blood did not fall below 20.80 volumes per cent. Knowing that anoxia is a potent cause of increase in flow of lung lymph, the animals in these experiments were given pure oxygen or 90 per cent oxygen plus 10 per cent carbon dioxide, so that anoxia should not enter the problem. A second point, already made, is that during the period of increased breathing cardiac output was practically unchanged. A third factor is the breathing itself when there is sudden resistance to inspiration. In this experiment, when the animal was compelled to breathe oxygen against resistance his minute volume fell to an average of 2.0 liters against 5.7 without resistance. It is clear from the figures upon blood oxygen that, owing to the use of 100 per cent oxygen for inhalation, anoxia did not occur. So far as can be seen, no essential factor affected lymph flow other than the increased negative pressure in the chest caused by breathing against resistance. There was no anoxia and cardiac output remained practically unchanged, but transudation occurred when the intrathoracic negative pressure was increased.

The experiments described thus constitute in the living animal a verification 1, of Graham's idea; 2, of the illuminating experiments of Brock and Blair, and 3, of the primitive but interesting observations of Yamada upon his docile Japanese soldiers.

In a second group of experiments lung lymph was collected during anoxia produced by breathing gas mixtures containing low amounts of oxygen.

In a previous paper (Warren and Drinker, 1942), it was shown that lymph collected from the lungs of dogs increased under such circumstances. In these animals the anterior mediastinum was opened, the upper part of the sternum being removed and a cannula introduced into one of the several lung lymphatics. The lymph collected does not represent the total outflow from the lungs, but it is a reliable cross-section of lymph drainage at any moment.

In this first paper (Warren and Drinker, 1942) it was brought out from the literature that anoxia does not increase cardiac output (Grollman, 1932; Doi,

1921), but no experiments were done in which cardiac output was actually measured when anoxia caused increase in lymph flow from the lungs. The point is far from academic. If, with anoxia, the output of the heart increases markedly, then a heightened production of lymph may mean nothing more than enhanced filtration of fluid from the lung capillaries as the result of a capillary bed, possibly widened, and subjected to at least a slight increase in pressure. If, on the other hand, anoxia is accompanied by greater flow of lung lymph and the cardiac output remains the same as in the control period, or falls, then, given absolutely constant lung movement during the entire experiment, it is fair to attribute increased lymph flow to increased permeability of the lung capillaries arising from anoxia.

Experiments on low oxygen. In dogs anesthetized with sodium barbital and under artificial respiration the anterior mediastinum was opened and a lung lymphatic cannulated. Systemic blood pressure was taken from the femoral artery with a mercury manometer. Rate of lymph flow was measured by collecting lymph for a given period of time into weighed tubes which were then reweighed. Cardiac output was determined by utilizing the direct Fick principle, the samples of mixed venous blood being taken through a glass tube introduced into the right auricle through the jugular vein.

Figure 3 will suffice to show the results obtained. In this case artificial respiration with air was used first. After the initial high lymph flow which invariably follows the procedure of cannulation of lung or other lymphatics, due to temporary obstruction during operative procedures, the lymph flow became very steady at about 9.7 mgm. per minute. The blood pressure, as is usual in such experiments, became constant, and cardiac output at the close of the period of ventilation with room air was 2.13 liters per minute. Ventilation was then shifted to a mixture of 13.5 per cent oxygen and 86.5 per cent nitrogen. Lymph flow and blood pressure remained unchanged, and the cardiac output was 1.92 liters per minute. After 1 hour and 35 minutes' administration of this mixture of gases, during which the arterial oxygen saturation fell from 16.08 to 9.48 volumes per cent, a second shift in ventilation was made, this time employing 10 per cent oxygen and 90 per cent nitrogen. Immediately the output of lung lymph rose, at first during a period when blood pressure was uniform with preceding values. The cardiac output, however, fell to 0.95 liter per minute and arterial oxygen saturation to 4.23 volumes per cent, when it became necessary to return to ventilation with room air. Obviously (fig. 3), as soon as this was done lymph flow from the lungs fell to normal level and was undisturbed in the final period of the experiment when a mixture of 10 per cent carbon dioxide and 90 per cent oxygen was used. During the recovery period, when 10 per cent oxygen plus 90 per cent nitrogen was replaced by room air, oxygen saturation of the arterial blood reached 14.53 per cent and cardiac output was 1.27 liters per minute.

This experiment and others of similar type indicate that the production of extravascular fluid in the lung parenchyma, which, in good part, is drained off as lung lymph, depends upon anoxia of the lung capillaries and not upon augmenta-

tions of pressure and flow of blood through the lungs. No one who approaches the problem of pulmonary edema can be surprised at this conclusion. The normal mammal can experience tremendous increases in pulmonary blood flow during exercise. The vascular bed in the lungs is so huge and so distensible that significant increases in pulmonary blood pressure do not occur unless return of blood to the left ventricle is impeded. If one obstructs the pulmonary veins in an animal with a normal heart, and then gives adrenin or ephedrine so as to drive
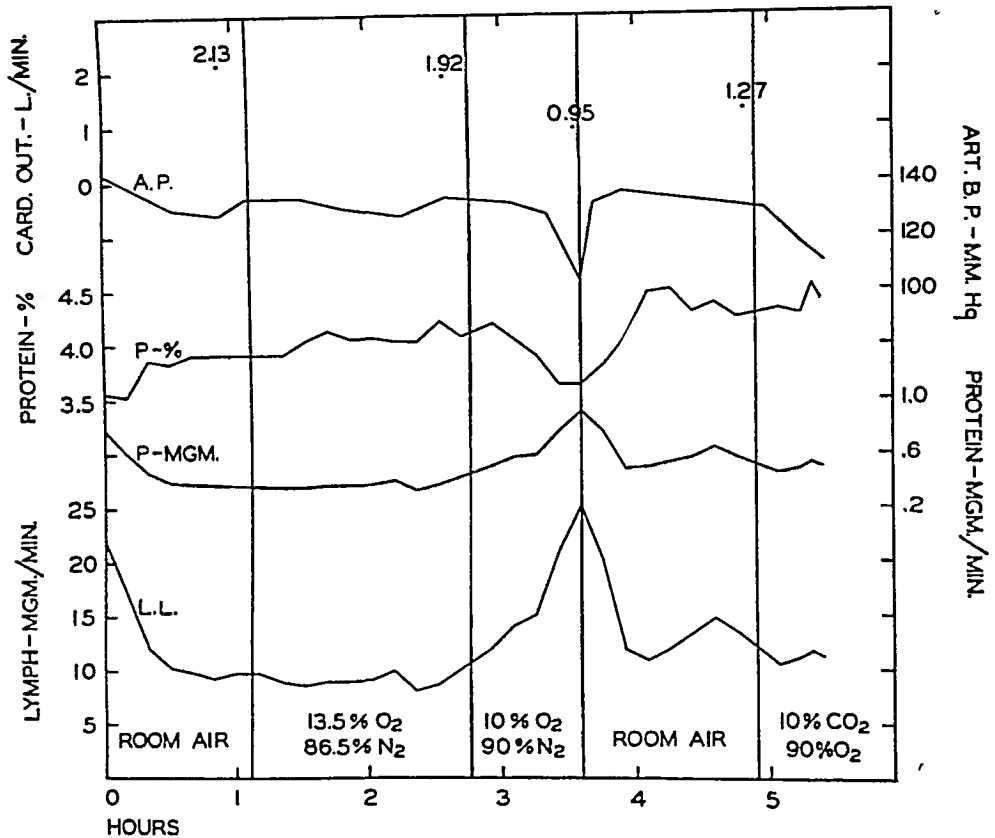
Fig. 3. Effect of lowering alveolar oxygen. *Dots with figures*, cardiac output in liters per minute; *upper curve*, A.P., arterial blood pressure; *second curve*, P-%, protein in lung lymph in per cent; *third curve*, P-MGM., protein in lung lymph in milligrams per minute; *fourth curve*, L.L., milligrams of lung lymph per minute. *Ordinates*, as designated; *abscissae*, time in hours. Between the vertical lines, animal breathed gas mixtures as designated. Rate and stroke of respiratory pump uniform throughout.

up cardiac output, the pulmonary blood pressure can show short but surprising rises. In the experiment we have reported there has been no hindrance of return of blood to the heart through the lungs, and the output of the heart has fallen rather than risen during the period of anoxia. The conclusion is unescapable that oxygen lack alone has caused the lung capillaries to leak abnormally, and that as soon as extreme anoxia is relieved by ventilation with room air this increase in permeability ceases.

The effects of oxygen in maintaining the lung capillaries at normal permeability cannot be over-emphasized, and the subtlety with which fluid leaks from the capillaries into the lung parenchyma should be one of the ever-present nightmares of clinicians. If, to anoxia, as in cases of cardiac decompensation, increased pressure in the pulmonary capillaries is added, lung edema will occur with even greater rapidity and more malign results.

SUMMARY

1. The anatomy of the lung lymph drainage in the dog is described, and it is made clear that lung and heart lymph enter the blood through the right lymphatic duct.

2. It is held that cardiac activity, measured through output, remaining steady, fluctuations in lymph flow from the right duct reflect changes in the production and flow of lung lymph.

3. Experiments are described which show that when an animal breathes against resistance so as to increase intrathoracic negative pressure, the flow of lymph from the lungs increases; and if the resistance to inspiration is very high, there may be not only a gain in the flow of lung lymph but the fluid may actually become bloody.

4. These facts indicate the influence of the extravascular negative pressure in producing transudation of fluid from the lung capillaries into the lung parenchyma.

5. Further experiments are described in which, under absolutely uniform artificial respiration, expiration being without suction, dogs have been subjected to progressive severe anoxia.

6. When the oxygen in the mixture used for artificial respiration was 13.5 per cent, no increase in the flow of lung lymph was noted.

7. On reducing the oxygen to 10 per cent, a sudden increase in the flow of lung lymph occurred, which ceased promptly on returning to ventilation with air.

8. The changes in the production and flow of lung lymph during anoxia cannot be attributed to increased cardiac output or extension of the filtering bed of the lung capillaries, since the output of the heart fell markedly during anoxia. Consequently it must be concluded that when sufficient oxygen is not available the lung capillaries promptly become abnormally permeable, but if this condition is not allowed to go too far they readily return to normal by ventilation with adequate oxygen.

REFERENCES

BROCK, R. C. AND E. A. BLAIR.   J. Thoracic Surg. 1: 50, 1931.
GRAHAM, E. A.   J. A. M. A. 76: 784, 1921.
GROLLMAN, A.   The cardiac output of man in health and disease.   Charles C. Thomas, Springfield, Ill. and Baltimore, Md., 1932.
ROUVIÈRE, H.   Anatomie des lymphatiques de l'homme.   Masson et Cie, Paris, 1932.
SAPPEY, P. C.   Anatomie, physiologie, pathologie des vaisseaux lymphatiques considérés chez l'homme et les vertébrés.   Adrien Delahaye, Paris, 1874.
WARREN, M. F. AND C. K. DRINKER.   This Journal 136: 207, 1942.
YAMADA, S. AND ASSISTANTS.   Ztschr. ges. exper. Med. 90: 342, 1933.
DOI, Y.   J. Physiol. 55: 43, 1921.

VOLUME OF AIR MOVED BY ARTIFICIAL RESPIRATION IN ANESTHETIZED MEN

D. R. HOOKER, W. B. KOUWENHOVEN AND J. ARTHUR YORK

From the School of Hygiene and Public Health, the School of Engineering and the Department of Surgery of the Johns Hopkins University

An earlier paper reported (1) results of the measurement of air moved by application of artificial respiration to conscious men. The primary purpose of that study was to evaluate the worth of a special method of artificial respiration, the Pole-top Method, by comparing its efficiency with the Schaefer Method. The latter method is designed for subjects lying prone. The Pole-top Method is designed for subjects in the trunk-vertical position and is of recent development (2) to meet a particular need, i.e., for application to men servicing electric power lines who may suffer "electric shock" and therewith respiratory paralysis as the result of contact with high voltage circuits.

These workers support themselves on the power-line poles by safety straps and when they are injured considerable time must elapse before they can be lowered to the ground. This time factor may well be of significance in the successful application of the older methods of artificial respiration. The Pole-top Method was developed to overcome this delay. It is applied by a fellow-worker who, supporting the victim on his own safety-strap in the trunk-vertical position (in effect holding the victim in his lap), spreads his hands over the lower abdomen and makes vigorous rhythmical upward lifts, thus forcing air to be expired. Further detailed description of the procedure may be found elsewhere (1, 2, 3).

Our earlier study, made on conscious men, indicated that the volume of air moved by the Pole-top Method when applied to subjects in the trunk-vertical position is somewhat greater than that moved by the Schaefer Method as applied to the same subjects in the prone position. Although we used only intelligent subjects presumably capable of relaxed passivity the results secured are open to criticism because the subjects may have co-operated unintentionally.

It thus seemed desirable to repeat the observations on anesthetized subjects in whom there was no possibility of co-operative aid. One of the barbiturates, sodium pentothal, was administered intravenously. The administration of this drug promptly effects unconsciousness and complete muscular relaxation. At the outset respiratory activity is depressed but it shortly returns to a fairly steady state which, by judicious administration of repetitive doses, may be safely maintained for a considerable period of time.

The data on the three subjects who were carried through the experimental procedure successfully are given in table 1. This comprises the detailed results secured when they were conscious as well as unconscious, together with their averages and the averages of the fifteen conscious subjects previously studied. Table 2 summarizes these results, the values being expressed in percentage in-

crease of air moved during application of artificial respiration over that of the corresponding control period of natural breathing.

TABLE 1

Respirations per minute and air moved per respiration in natural breathing and during artificial respiration applied before and after induction of anesthesia, together with the averages on fifteen conscious subjects previously reported

CONDITIONS	A 28 YRS. 139 LBS.	B 38 YRS. 160 LBS.	C 38 YRS. 167 LBS.	AVERAGE A, B, AND C	AVERAGE 15 SUBJECTS
Conscious					
Natural breathing in prone position:					
Resp. p. Min.	15	14	11	13	13
Cc. p. Resp.	375	625	770	590	545
Original Schaefer artificial respiration:*					
Resp. p. Min.	14	10	12	12	11
Cc. p. Resp.	424	1000	1111	845	868
Modified Schaefer artificial respiration:**					
Resp. p. Min.	13	11	10	11	11
Cc. p. Resp.	457	1250	1250	986	1028
Natural breathing in trunk-vertical position:					
Resp. p. Min.	14	16	10	13	15
Cc. p. Resp.	413	625	1000	679	558
Pole-top artificial respiration:					
Resp. p. Min.	10	14	10	11	10
Cc. p. Resp.	875	1111	1111	1032	1363
Unconscious					
Natural breathing in prone position:					
Resp. p. Min.	17	18	19	18	
Cc. p. Resp.	172	384	237	264	
Original Schaefer artificial respiration:*					
Resp. p. Min.	11	11	12	11	
Cc. p. Resp.	430	714	625	590	
Modified Schaefer artificial respiration:**					
Resp. p. Min.	9.5	10	12	10	
Cc. p. Resp.	571	910	768	746	
Natural breathing in trunk-vertical position:					
Resp. p. Min.	21	17	19	19	
Cc. p. Resp.	170	367	280	272	
Pole-top artificial respiration:					
Resp. p. Min.	11	12	13	12	
Cc. p. Resp.	714	912	770	799	

* Hands held in position between pressure strokes.
** Hands allowed to slip off at the end of each pressure stroke.

These findings, of course, are not strictly comparable to what might be expected to follow in individuals in whom all respiratory activity had ceased but they are probably as close as laboratory conditions can provide. They are fur-

thermore of interest in giving evidence of the relative efficiency value of the three methods of artificial respiration studied and, so far as we know, represent the first effort to evaluate the worth of artificial respiration in subjects under general anesthesia.

PROCEDURE. The procedure was much the same as in our earlier study, the same operator acting throughout, except the present subjects were not given preliminary training in the technique. The sequence of events was: 1. With mask and pneumograph in place the subject lay prone on the mat and rested five minutes. 2. Operator took position and waited two minutes. 3. Record of air moved in natural breathing. 4. Record of air moved by original Schaefer method (operator's hands held in position between pressure strokes). 5. Pause lasting two minutes. 6. Record of air moved by modified Schaefer method (operator's hands allowed to slip off at end of each pressure stroke). 7. Subject rose without displacing mask and assumed a trunk-vertical sitting position where he rested

TABLE 2

Raw data taken from table 1

Amount of air moved per minute and per respiration expressed as percentage increase over corresponding control period. The open figures represent the averages for the three subjects here reported; the bracketed figures represent the averages of the fifteen subjects previously reported. The right hand column of this table, as indicated by the heading, shows still another evaluation of the data.

	PERCENTAGE INCREASE OVER CONTROL PERIOD. AIR MOVED PER MIN.		PERCENTAGE INCREASE OVER CONTROL PERIOD. AIR MOVED PER RESPIRATION		PERCENTAGE DIFFERENCE IN AIR MOVED PER MINUTE BY ARTIFICIAL RESPIRATION WHILE ANESTHETIZED COMPARED TO NORMAL BREATHING WHILE CONSCIOUS
	Conscious	Anesthe-tized	Conscious	Anesthe-tized	
Original Schaefer artificial resp......	32 (35)	37	43 (59)	123	15% decrease
Modified Schaefer artificial resp.....	41 (60)	57	67 (89)	183	3% decrease
Pole-top artificial resp..............	29 (63)	86	52 (144)	194	9% increase

five minutes. 8. Operator took subject onto his safety-belt strap followed by another pause of five minutes. 9. Record of air moved in natural breathing (trunk vertical). 10. Record of air moved by Pole-top method. 11. Anesthesia induced followed by pause until steady state of respiratory activity obtained. 12. Record of air moved by Pole-top method (trunk vertical, subject unconscious). 13. Unconscious subject lifted down without displacing mask and placed prone on mat and rested five minutes. 14. Operator took position. 15. Record of air moved in natural breathing (subject unconscious). 16. Record of air moved by original Schaefer method (subject unconscious). 17. Pause lasting two minutes. 18. Record of air moved by modified Schaefer method (subject unconscious).

RESULTS. The raw data from the observations are presented in table 1. It will be noted that the imposed respiratory rate (during artificial respiration) is less than in natural breathing while the amount of air moved per respiration is

greater.   It is thus apparent that all three of the methods of artificial respiration employed are more than adequate to supply the requisite pulmonary ventilation.

In table 2 the foregoing data are used to express the results as percentage increases of the amount of air moved per minute and per respiration over the corresponding periods of natural breathing.   Here the Pole-top Method shows as inferior to the Schaefer Method on the three new subjects when conscious. These values, compared with the values estimated from the conscious subjects earlier reported (1) (bracketed figures) suggest that a psychological factor may be involved in working with conscious subjects.   Such a factor may, however, not make its appearance if the subjects are given preliminary training experience as was done in our earlier work.   However, when the same three subjects were unconscious the values are clean cut and consistent.

It is, furthermore, to be noted in table 2 that in every instance the values, both for air moved per minute and per respiration, are greater when the subjects were unconscious.

Table 2 also shows (right hand column) the average percentage difference in air moved per minute by the three methods of artificial respiration while the subjects were unconscious compared with natural breathing while conscious. Anesthesia, of course, tends to depress the metabolism.   Nevertheless it is of interest to note that the values presented are comparable to those shown in the other two columns of the table and tend to support the previous statement as to the relative efficacy of the methods tested.

SUMMARY

Data are presented on the amount of air moved by artificial respiration in three subjects when conscious and when unconscious (anesthetized).   These data indicate that: 1, more air is moved in the unconscious (anesthetized) than in the conscious subject with each (artificial) respiratory act; 2, the modified Schaefer Method (hands allowed to slip off at the end of each pressure stroke) is superior to the original Schaefer Method; 3, the Pole-top Method of artificial respiration, for the purpose intended, is an adequate and valuable procedure; 4, the results indicate that the Pole-top Method in the trunk vertical position is more efficacious than the Schaefer Method in the prone position.

REFERENCES

(1) KOUWENHOVEN, W. B., D. R. HOOKER AND O. R. LANGWORTHY.   Elec. Engineering 60: 41, 1941.
(2) OESTERREICH, E. W.   Penn. Electric Asso., March 1, 1939.
(3) DALZIEL, C. F.   Electrical West, April 1941.

THE RÔLE OF THE ADRENAL CORTEX IN PREVENTING HYPOGLYCEMIC CONVULSIONS

V. ARNETT, M. KESSLER AND E. GELLHORN[1]

From Departments of Physiology and Psychiatry, University of Illinois, College of Medicine, Chicago

The anti-insulin effect of hormones of the adrenal cortex and of the adrenotropic hormone of the anterior pituitary has been established by experiments of Grattan, Jensen and Ingle (1941) who showed that injection of these hormones may prevent insulin convulsions. However, these studies do not answer the question as to whether the physiological secretion of the adrenal cortex is adequate to modify the effects of insulin. The observation of Conn (1941) that hyperinsulinism in the human cannot be alleviated by injections of relatively large quantities of potent adrenocortical extracts suggests that the experiments of Grattan and collaborators are of a greater pharmacological than physiological significance. The experiments described in this paper were designed to throw some light on the physiological rôle of the adrenal cortex in insulin hypoglycemia.

METHODS. The experiments were performed on two groups of male rats (250 to 300 grams): one group was adreno-demedullated and the other was completely adrenalectomized. After a fast of 16 hours insulin (Lilly) was injected intraperitoneally and the blood sugar was determined after Hoffman (1937). The electroencephalogram (E.E.G.) was obtained by using Hoagland's method of inserting phonograph needles into the skull.

RESULTS. Table 1 shows the effects of 0.2 u. of insulin/kgm. injected intraperitoneally into adreno-demedullated and adrenalectomized rats. The lowering of the blood sugar is practically identical in both groups (table 1). There is, however, a marked difference in the frequency of cerebral symptoms as table 2 indicates. Symptoms in the form of coma or convulsions were observed in only 16 per cent of the adreno-demedullated animals although 76.5 per cent of the adrenalectomized animals showed these symptoms. The sensitivity of the latter group is further illustrated by eleven additional cases (not included in table 1) in which convulsions occurred so abruptly that blood samples could not be obtained. Inclusion of this group in table 2 raises the percentage of cerebral involvement to 84 in the adrenalectomized rats.

Since table 1 shows that convulsions do not depend solely upon the hypoglycemic level attained by the adreno-demedullated and adrenalectomized rats, it appeared to be likely that the rate of fall of the blood sugar might be the deciding factor. Comparison of the effect of insulin on 6 adrenalectomized and 9 adreno-demedullated rats shows that although the final blood sugar levels are similar the rate of fall is less rapid in the adreno-demedullated group.

It is worthy of note that the delayed fall of the blood sugar persisted when

653

insulin was injected into the adreno-demedullated rats in even greater amounts (0.3 to 0.5 u/kgm.) than were administered to the adrenalectomized group (0.2 u/kgm.). From these experiments it follows that the rate of fall of blood sugar is delayed by the hormones of the adrenal cortex. This effect of the adrenal cortex can be easily overcome in adreno-demedullated animals by injecting very large quantities of insulin. When 5 u/kgm. are injected, coma and convulsions occur in nearly all animals within 40 to 60 minutes. Apparently the insulin is circulating in the blood in concentration sufficiently high to offset the anti-insulin effects of the adrenal cortex. Therefore, the rate of the fall in blood sugar as well as the cerebral symptomatology becomes similar to that of adrenalectomized rats.

TABLE 1

Effect of 0.2 unit insulin (Lilly) intraperitoneally on the blood sugar of rats

NUMBER OF ANIMALS	TYPE OF OPERATION	INITIAL BLOOD SUGAR	BL. SUGAR AFTER 60 MIN.
		mgm.%	mgm.%
25	Adrenodemedullation	98 ± 4.5	74 ± 9.1
22	Adrenalectomy	96 ± 6.0	70 ± 9.9

TABLE 2

Cerebral symptoms following the injection of 0.2 u. insulin

NUMBER OF ANIMALS		TYPE OF SYMPTOMS	PER CENT OF SYMPTOMS
25	Adrenomedullated	2 comas 2 convulsions	16
33	Adrenalectomized	7 reflexes depressed 9 comas 12 convulsions	84

All of the data presented thus far indicate that the difference between the two groups with respect to cerebral symptoms cannot be explained on the basis of hypoglycemia alone. It seems of importance to prove this point more specifically. Table 3 shows how adreno-demedullated and adrenalectomized animals react at various hypoglycemic levels. It is obvious from the table that cerebral symptoms occur in adrenalectomized animals at relatively high blood sugar levels. None of the adreno-demedullated animals shows an involvement of the central nervous system at comparable levels. Moreover, at blood sugars between 66 and 58 mgm. per cent all adrenalectomized animals show coma or convulsions whereas in the adreno-demedullated groups 50 per cent of the animals remain normal.

This difference in the reactivity of the brain at low blood sugars is brought out in figure 1 in which the adrenalectomized animal reacts with coma characterized by the disappearance of alpha waves and the appearance of delta potentials.

The adreno-demedullated animal reaches a far lower degree of hypoglycemia without showing any change in E.E.G. or gross behavior.

TABLE 3

Relation of hypoglycemic level to cerebral symptoms

BLOOD SUGAR	I. Adrenodemedullated rats			BLOOD SUGAR	II. Adrenalectomized rats		
mgm.%				mgm.%			
74	6 normal			77–80	6 normal	2 comas	
70	13 normal			74	1 normal	1 depressed	1 coma
66	6 normal	1 depressed reflexes	2 comas	70	2 normal	4 depressed	1 coma
62	3 normal	1 depressed reflexes	1 coma	66	1 coma		
58	1 normal	2 depressed reflexes	2 comas	62	5 comas	2 convulsions	
				58	5 convulsions	1 coma	
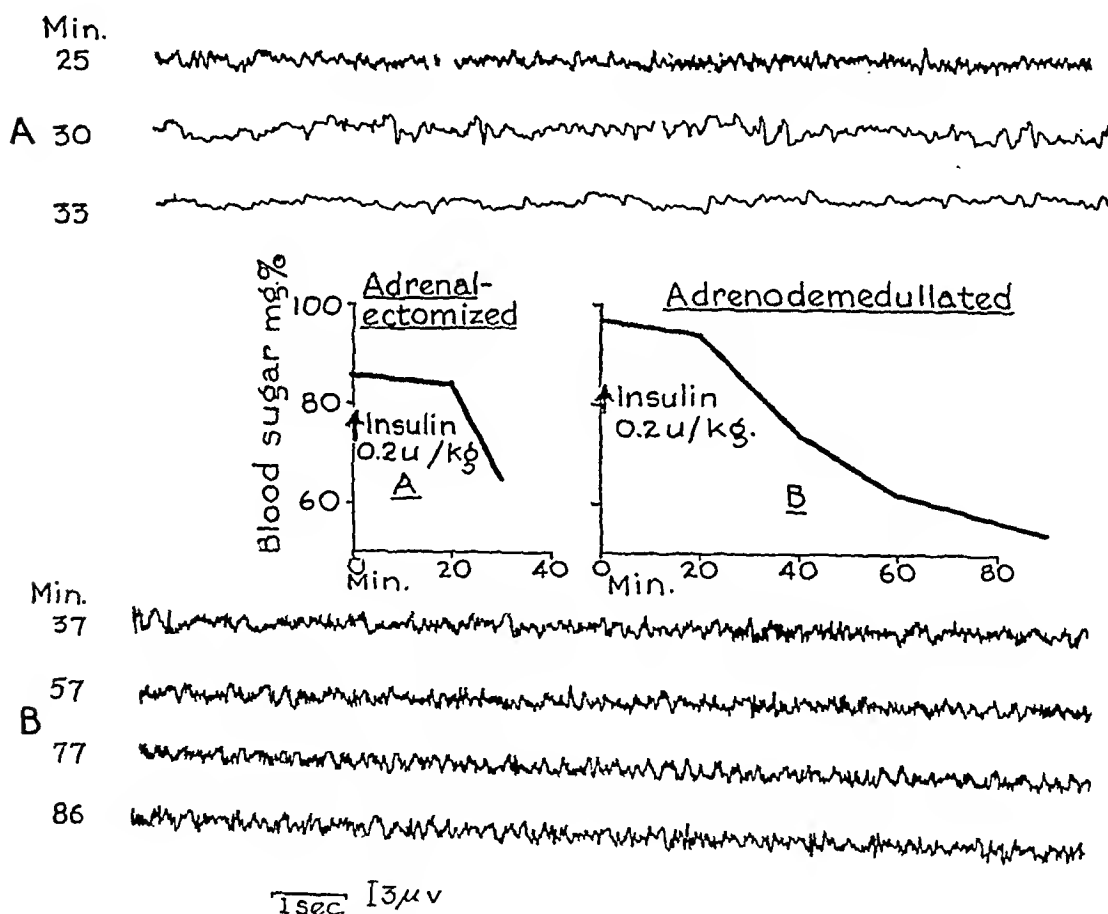
Fig. 1. A. Adrenalectomized rat; 0.2 u. insulin/kgm. intraperitoneally. Effect on blood sugar and E.E.G.

B. Adrenodemedullated rat; same as above.

DISCUSSION. Our observations on cerebral symptoms in adrenalectomized and adreno-demedullated animals confirm similar studies of Zucker and Berg

(1937). However, the reactivity of the blood sugar is different in the experiments of these authors and our own. Zucker and Berg observed a delay in the recovery of the blood sugar of their adrenalectomized dogs but saw no differences in the rate of fall. It should be mentioned however that these authors used dogs and maintained the animals with adequate quantities of cortical extracts. Our adrenalectomized rats were not given any hormones but kept in good condition solely by the ingestion of 2 per cent NaCl.

Since in our earlier work (Gellhorn, Feldman and Allen, 1941) adreno-demedullated animals often showed coma when injected with 0.2 u. insulin/kgm. it seems probable that these animals suffered from adreno-cortical deficiency. This interpretation is supported by the fact that the adreno-demedullated animals discussed in the present study showed a progressive loss of weight when given 2 per cent NaCl instead of water, whereas in the earlier work they remained in good condition on the salt diet.

The blood sugar curves presented in this paper indicate that the cortical hormones delay the action of insulin in the adreno-demedullated group. They also show that the frequency of cerebral symptoms is not due to the blood sugar level. Assuming that the symptoms occur when the utilization of sugar reaches a certain low value, it appears most probable that the glucose uptake by the brain is higher in the adreno-demedullated group than in the adrenalectomized group at similar blood sugar levels. This suggests that the brain circulation is better in the adreno-demedullated than in the adrenalectomized animals.

Circulatory disturbances in adrenalectomized animals are likewise suggested by the observation that they frequently fail to recover from insulin convulsions on injection of glucose. The absorption of glucose from the peritoneal cavity of these animals is prompt as indicated by sugar measurements. Nevertheless, the rats often remain in a comatose condition. Whether this is due to inadequate brain circulation or whether hypoglycemia induces irreparable brain damage relatively quickly in adrenalectomized animals cannot be decided at the present time. It is, however, more probable that circulatory failure plays the predominant rôle since adrenalectomy predisposes to shock (Swingle, Parkins and Remington, 1941).

SUMMARY

1. The effect of 0.2 u. insulin/kgm. on adreno-demedullated and adrenalectomized rats is similar as far as the minimal blood sugar level is concerned but the rate of fall is more rapid in the adrenalectomized animals.

2. Coma and convulsions occur frequently in the adrenalectomized and rarely in the adreno-demedullated group in spite of similar degrees of hypoglycemia. This difference is seen not only in the gross behavior but also in the E.E.G. which may be normal during hypoglycemia in an adreno-demedullated animal although the same blood sugar level invariably causes coma, disappearance of alpha and appearance of delta potentials in adrenalectomized animals.

REFERENCES

CONN, J. W. AND E. S. CONN. Arch. Int. Med. **68**: 1105, 1941.

GELLHORN, E., J. FELDMAN AND A. ALLEN. Endocrinology **29**: 137, 1941.

GRATTAN, J. F., H. JENSEN AND D. J. INGLE. This Journal **134**: 8, 1941.

HOFFMAN, W. S. J. Biol. Chem. **120**: 51, 1937.

SWINGLE, W. W., W. M. PARKINS AND J. W. REMINGTON. This Journal **134**: 503, 1941.

ZUCKER, T. F. AND B. N. BERG. This Journal **119**: 539, 1937.

RENAL EXCRETION OF SULFATE[1, 2, 3]

BERNARD M. SCHWARTZ, PAUL K. SMITH AND ALEXANDER W. WINKLER

From the Department of Internal Medicine and the Laboratory of Pharmacology, Yale University School of Medicine, New Haven

For a large number of organic filterable solutes, such as the foreign sugars, the urinary excretion rate is in general proportional to the serum concentration. Under specified conditions the ratio of these two quantities, the clearance, is a constant independent of the serum concentration. Such clearances are closely dependent on the glomerular filtration rate and may indeed for some substances be numerically equal to it. The clearance of each substance is largely independent of the presence or absence of other substances in the urine.

Clearances of ions may be calculated in the same way as those of the organic filterable substances, but their behavior is less simple. In the first place, these clearances are not independent of serum concentration. In the second place, the virtual electroneutrality of the urine means that the sum of the concentrations of cations must always equal that of anions. With each cation an equivalent amount of anion must be excreted, and vice versa. It is manifestly not possible for the excretion of all anions and cations to depend simply on their respective concentrations in serum.

Nevertheless excretion of electrolytes does proceed in an orderly fashion. The experiments to be described attempt to analyze the relationships between electrolyte excretion and certain other variables, in order to formulate the type of general statements which can be made concerning ionic excretion. Sulfate was chosen as a reference substance, partly because of its representative character, partly because its concentration in serum may safely be increased to a greater extent than concentrations of other ions. Excretion of inorganic sulfate following the intravenous injection of various sulfate salts has been compared with the concentration of sulfate in serum, with glomerular filtration, and with the excretion of associated cations and anions.

MATERIALS AND METHODS. Adult female dogs were used in all experiments. An inlying catheter was kept in place, and after a preliminary period of urine collection, a solution of the salt to be studied was injected intravenously. The exact composition and concentration of the solution injected in each experiment is indicated in the tables. The urine specimens during the infusion and during several subsequent collection periods were saved, completeness of collection

being insured by washing out the bladder with water. The exact time at which each period began or closed was recorded. Samples of blood were obtained from the femoral vein, both before the infusion and at the beginning and end of each urine collection period thereafter. Creatinine, 10 grams in 100 cc. of solution, was injected intraperitoneally just before the salt infusion. Urine and serum samples were analyzed chemically for sulfate, creatinine and various associated cations and anions. Clearances were calculated for each period by dividing the average excretion rate for that period by the mean serum concentration during the period. The latter was determined by the method of Winkler and Parra, which has been previously described (16). Creatinine clearance is assumed to be identical with glomerular filtration rate in the dog (11, 12).

Sodium was determined by the method of Hald (7), sulfate by the method of Cope (1), and chloride by the method of Van Slyke (10). Magnesium and creatinine were determined by methods previously described (14, 16).

RESULTS. In table 1 are presented the results of experiments in which sulfate, alone and in combination with sodium chloride, was injected. In tables 2 and 3 are presented the results of similar experiments in which potassium sulfate and magnesium sulfate, respectively, were employed. The results will be analyzed in several ways.

A. *Relationship between urinary excretion of sulfate and serum concentration of sulfate in all experiments.* In figure 1 the excretion rate of sulfate is plotted against serum concentration in experiments of table 1. Figure 2 is a similar plot showing the results of the experiments of tables 2 and 3. There is evidently considerable variation from experiment to experiment in the exact relationship of excretion rate to serum concentration. Nevertheless, from these figures it appears that there is in general for each experiment a linear relation between the rate of excretion of exogenous sulfate and its concentration in serum. Extrapolations of these lines do not pass through the origin, but intersect the abscissa at various points. The lines of figure 2, representing the excretion of moderate amounts of exogenous sulfate, tend to intersect near the same point, corresponding to some 3 mEq. per liter. Only sodium sulfate could be used to study the excretion of large amounts of sulfate, since the salts of magnesium and of potassium are too toxic. The lines (fig. 1) are steeper than those of figure 2, and when extrapolated cut the abscissa at higher concentrations. It is noteworthy that this linearity held good in spite of the fact that the simultaneous creatinine clearances varied widely (table 1).

B. *Associated excretion of cations.* (1) *Sodium sulfate experiments.* In the four experiments in which sodium sulfate alone was injected, the concentration of sodium in urine at first almost exactly equalled that of sulfate. In subsequent periods the concentration of sodium was usually a little lower than that of sulfate. In experiment 13 of table 1 potassium excretion increased sufficiently to maintain substantial equality in the sums of anions and of cations. The main associate of the sulfate was always sodium, however.

In two of the three experiments in which mixtures of sodium sulfate and sodium chloride were given the quantitative parallelism between urinary sodium

TABLE 1

Intravenous injection of hypertonic solution of sodium sulfate and sodium chloride, singly and in combination

EXPERIMENT NO. DOG WEIGHT, KGM.* SALT GIVEN, mM.**	PERIOD†	DURATION, MINUTES	URINE FLOW, CC. PER MINUTE	CONCENTRATION, m.Eq. PER LITER — Urine NH₄+	K+	Na+	SO₄⁻	Cl⁻	Serum Na+	SO₄⁻	Cl⁻	CLEARANCE, CC. PER MINUTE Na+	SO₄⁻	Cl⁻	Creatinine
No. 6 25.0 kgm. A Na₂SO₄ 206mM.									142.4	2.7					
	1	30	0.23			3	—	—				0.0			
	2	30	15.00			221	—	9							
									177.5	65.3					
	3	20	12.90			244	244	4				18.0	54		83
									175.5	51.5					
	4	30	7.00			282	309	1				12.0	50		83
									164.5	36.0					
	5	60	2.83			289	365	1				5.1	36		55
									158.0	22.1					
	6	65	1.28			259	363	2				2.1	23		32
									157.4	17.6					
	7	125	1.05			209	293	9				1.4	24		32
									155.3	8.9					
No. 7 24.7 kgm. A Na₂SO₄ 103 mM. + NaCl 103 mM.									139.2	2.3	105.9				
	1	42	0.55			15	16	38				0.0	4	0.2	
	2	39	8.40			188	179	31							
									172.0	29.3	108.4				
	3	33	5.94			229	206	23				8.1	51	1.3	119
									163.5	19.9	110.3				
	4	68	2.18			244	289	6				3.2	44	0.1	78
									164.2	10.6	110.7				
	5	87	0.86			160	293	4				0.8	31	0.0	57
									165.0	6.5	111.6				
No. 8 24.6 kgm. A Na₂SO₄ 154 mM. + NaCl 308 mM.									146.1	2.3	102.7				
	1	25	0.60			2	28	3				0.0	7	0.0	
	2	48	9.80			215	151	624							
									189.8	38.7	118.9				
	3	54	8.43			276	221	536				12.6	62	3.7	64
									181.0	23.2	123.0				
	4	125	3.56			255	251	32				5.1	60	0.9	55
									175.6	9.6	125.7				
	5	144	0.92			202	232	9				1.1	36	0.1	53
									177.0	3.7	129.7				
	6	1025	0.75			194	31	132							
									144.4	3.5	113.2				
	7	1725	0.57			149		137							
No. 9 25.4 kgm. A NaCl 308 mM.									144.8		107.4				
	1	—	—												
	2	45	3.16			173		157							
									167.8		129.0				
	3	54	1.76			177		150				1.9		2.1	89
									156.2		127.4				
	4	89	1.19			166		155				1.2		1.5	67
									156.1		124.2				
	5	158	1.23			120		138				1.0		1.4	84
									153.0		121.6				
	6	1084	0.54			65		65				0.2		0.3	
									149.2		119.7				
	7	1440	0.79			100		85							

* Letters refer to individual animals.

** The total amount of salt given was dissolved in one liter, except in experiment 13, in which the volume of the infusion was two liters.

† Infusions given during period 2, except as otherwise noted.

TABLE 1—*Concluded*

Experiment No. / Dog weight / Salt given	Period	Duration, minutes	Urine flow, cc per minute	Urine NH₄⁺	Urine K⁺	Urine Na⁺	Urine SO₄⁼	Urine Cl⁻	Serum Na⁺	Serum SO₄⁼	Serum Cl⁻	Clearance Na⁺	Clearance SO₄⁼	Clearance Cl⁻	Clearance Creatinine
No. 10	1	19	0.11	264		27	46	11	141.3	2.2	104.4				
19.5 kgm. B	2	38	14.17	0		236	237	24				0.0	2	0.0	
Na₂SO₄ 206 mM.	3	31	7.55	2		297	305	13	176.2	64.7	90.8	13.2	39	1.1	58
	4	57	4.32	49		367	391	1	162.3	52.8	96.3	10.0	41	0.0	84
	5	121	1.20	13		443	554	3	160.5	30.2	101.3	3.4	39	0.0	60
	6	150	0.68	29		407	304	7	149.7	7.8	105.0	1.9	40	0.0	40
	7	990	0.20	—		125	90	25	143.7	3.0	107.3				
No. 11	1	71	0.11			91	82	191	142.0	2.0	98.4				
19.3 kgm. B	2	47	14.10			228	238	87				0.0	2	0.0	
Na₂SO₄ 137 mM. +	3	44	7.27			301	110	111	184.8	26.4	125.9	12.0	38	6.4	71
NaCl 410 mM.	4	55	2.47			313	170	87	176.2	15.9	126.3	4.4	28	1.6	59
	5	139	0.99			267	254	54	174.0	14.1	130.7	1.5	19	0.4	62
	6	1174	0.39			287	49	169	172.6	12.8	137.4				
	7	1320	0.29				35	176							
No. 12	1	—	—			119	0	121‡	142.5	2.3	92.1§				
24.2 kgm. C	2	44	3.60			143	0	110‡	135.0	2.3	87.3§	3.7	0	4.4	75
NaBr 154 mM. before period 1	3	53	8.72			266	278	10‡	174.6	51.4	73.5§	—	—	1.1	64
Na₂SO₄ 206 mM. during period 3	4	63	5.97			326	338	7‡	156.2	29.1	83.9§	12.0	50	0.6	57
No. 13	1	24	0.29	—	—	157	58		145.0	—		0.3			
26.0 kgm. D	2	36	5.75	4	34	301	331								
Na₂SO₄ 309 mM.	3	40	13.30	1	35	312	337		187.7	90.0		23.1	64		126
	4	63	6.90	1	41	335	401		171.2	54.8		14.1	70		117
	5	92	2.96	11	46	348	417		157.7	28.5		6.7	59		88
	6	80	1.34	13	62	381	434		151.7	15.5		3.3	50		62
									152.3	8.6					

‡ Bromide concentrations 9.8, 13.3, 1.5, and 0.5 mM per liter urine respectively in these four periods.
§ Bromide concentrations 16.1, 16.0, 16.1, and 14.5 mM per liter serum respectively in these four samples.

and sulfate is also quite close (expts. 7 and 8 of table 1). Initially the sodium excretion exceeded the sulfate excretion, enough chloride being excreted to make up the difference. In subsequent periods chloride excretion is almost wholly

suppressed, and sodium and sulfate concentrations are nearly equal. In the exceptional experiment (no. 11) chloride excretion continues in considerable amounts during and for some time after the infusion. The sum of chloride and sulfate concentrations first exceeds and then falls somewhat below that of sodium.

(2) *Potassium sulfate experiments.* In the two potassium sulfate experiments the rate of excretion of potassium falls much below that of sulfate. In experiment 15 of table 2 the difference is evidently almost exactly made up by the sodium, the excretion rate of which is considerably above normal.

TABLE 2

Intravenous injection of isotonic potassium sulfate solution

EXPERIMENT NO. DOG WEIGHT, KGM. SALT GIVEN, mM.	PER-IOD*	DURA-TION, MINUTES	URINE FLOW, CC. PER MINUTE	CONCENTRATION, m.Eq. PER LITER						CLEARANCE, CC. PER MINUTE		
				Urine				Serum				
				Na^+	K^+	SO_4^-	Cl^-	K^+	SO_4^-	K^+	SO_4^-	Crea-tinine
No. 14 25.2 kgm. K₂SO₄ 51 mM.	1	32	0.19		42	68	11	4.3	2.3	2	6	
	2	75	2.61		82	219	8					
	3	72	0.94		188	269	1	7.9	11.7	26	28	80
	4	52	0.40		138	220	5	6.3	7.4	9	12	46
	5	144	0.29		145	214	24	6.5	7.0	6	10	44
								7.4	6.0			
No. 15 16.0 kgm. K₂SO₄ 25.5 mM.	1	21	0.38	90	62	60	74	5.5	2.7			
	2	49	2.08	118	128	242	7					
	3	59	0.98	125	165	280	3	8.1	9.4	19	37	76
	4	89	0.39	75	165	223	6	8.6	4.6	8	24	70
	5	45	0.33	58	177	132	6	6.7	2.9	9	15	63
								6.3	2.9			

* Salt solution injected during period 2.

(3) *Magnesium sulfate experiments.* In the five magnesium sulfate experiments (table 3) there is a close parallelism between sulfate and magnesium in the urine, but the magnesium concentration is always distinctly less than that of sulfate. Chemical analyses for other cations and anions were not done.

C. *Associated excretion of anions.* (1) *Effects of sulfate on chloride excretion.* The injection of sodium or of potassium sulfate evidently virtually abolished the normal excretion of chloride for some time (table 1, expts. 6 and 10, table 2, expts. 14 and 15). A similar inhibition is however present even when sodium chloride is injected simultaneously with the sodium sulfate (table 1, expts. 7, 8

TABLE 3

Intravenous injection of isotonic magnesium sulfate solution

EXPERIMENT NO. DOG WEIGHT, KGM. SALT GIVEN, mM.	PERIOD*	DURA- TION, MINUTES	URINE FLOW, CC. PER MINUTE	CONCENTRATION, m.Eq. PER LITER				CLEARANCE, CC. PER MINUTE		
				Urine		Serum				
				Mg++	SO₄	Mg++	SO₄	Mg++	SO₄	Creati- nine
						1.9	2.8			
No. 1 17.4 kgm.	1	24	0.42	9	16			2	2	
	2	24	3.50	122	171					
						15.2	14.5			
MgSO₄ 27.7 mM.	3	33	1.60	168	232			23	34	56
						9.2	9.0			
	4	46	1.50	130	179			24	36	64
						6.8	6.4			
No. 2	1	38	0.16	6	11	1.4	3.4	1	0	
	2	32	0.22	334	464					
						14.2	15.8			
18.8 kgm.	3	40	1.20	199	284			20	27	46
						11.1	10.9			
MgSO₄ 24.8 mM.	4	60	1.30	140	190			22	27	49
						6.0	7.5			
	5	1024	0.40	11	57					
						1.2	3.0			
No. 3 11.7 kgm.	1	52	0.29	7	23			2	2	
	2	24	1.40	163	217					
						13.7	13.5			
MgSO₄ 18.0 mM.	3	71	0.49	307	385			15	21	42
						7.4	6.8			
	4	71	0.27	260	338			12	16	41
						4.9	4.6			
	5	96	0.28	138	185			10	13	39
						3.3	3.7			
						1.8	2.6			
No. 4 30.3 kgm.	1	25	0.40	7	14					
	2	35	1.10	211	368					
						13.7	12.7			
MgSO₄ 38.5 mM.	3	48	1.40	164	270			20	36	53
						9.6	8.6			
	4	100	0.97	140	206			16	29	3S
						7.5	5.5			
						1.4	3.1			
No. 5 18.9 kgm.	1	19	0.11	—	6				0	
	2	35	1.70	160	185					
						12.1	13.4			
MgSO₄ 25.3 mM.	3	145	0.72	226	311			17	21	46
						8.0	8.3			
	4	68	0.41	193	222			12	13	33
						6.0	6.1			
	5	1085	0.35	192	241			13	15	36
						4.6	5.0			

* Salt solution injected during period 2.

Figures 1-5

and 11). In these experiments chloride excretion diminishes to virtually nil in spite of a progressive elevation of the chloride concentration in serum and the presence of an excess of sodium chloride in the body requiring excretion. Experiment 9 proves that this inhibition of chloride excretion is related to the simultaneous presence of sulfate, since injection of sodium chloride alone is followed by an increased and approximately parallel excretion of both sodium and of chloride.

(2) *Effects of anions on sulfate excretion.* In figure 1 urinary excretion of sulfate in three separate experiments using the same animal is compared with its serum concentration in three separate experiments (6, 7 and 8 of table 1). (These three experiments are represented by solid lines.) The line furthest to the right (6) represents the excretion after the injection of sodium sulfate alone, the middle line (7) the excretion after injection of a mixture of two parts of sodium sulfate and three parts sodium chloride, while the line furthest to the left (8) represents the excretion after an infusion of a mixture of two parts of sodium sulfate and four parts of sodium chloride. The greater the proportion of sodium chloride given, the greater the excretion rate of sulfate corresponding to any given serum concentration. In other words, increasing the proportion of chloride in the infusion increased the clearance of sulfate. This appears to be a clear effect of an associated anion upon sulfate excretion.

D. *Relation of sulfate excretion to glomerular filtration rate.* In figure 3 creatinine clearance is plotted against simultaneous sulfate clearance for each experiment. A certain general parallelism is evident, but the dispersion of the points is considerable. In figure 4 the ratio of sulfate to simultaneous creatinine clearance is plotted against serum sulfate. No definite relationship is found. This means that the *proportion* of filtered sulfate which is reabsorbed by the tubules is not necessarily greater at high than at low concentrations of sulfate. This is at variance with the findings of Goudsmit, Power and Bollman (6); possible reasons for the differing result will be discussed elsewhere. It has been noted above that in figure 1 the lines relating serum concentration to urinary excretion appear straight, although glomerular filtration varied considerably in each experiment. This suggests that under these particular conditions excretion rate was but little affected by variation in glomerular filtration.

Fig. 1. Relationship between sulfate excretion rate and concentration of sulfate in serum following the injection of sodium sulfate alone and in combination with sodium chloride. Numbers refer to individual experiments of table 1. The solid lines represent three experiments on the same animal, in which differing proportions of sodium chloride to sodium sulfate in the infusion were employed. Other experiments are represented by broken lines.

Fig. 2. Relationship between sulfate excretion and concentration of sulfate in serum following the injection of magnesium sulfate and of potassium sulfate.

Fig. 3. Relationship between sulfate clearance and creatinine clearance.

Fig. 4. Relationship between the ratio of sulfate to creatinine clearance and the concentration of sulfate in serum.

Fig. 5. Relationship between sodium excretion rate and the concentration of sodium in serum. Numbers refer to individual experiments of table 1.

DISCUSSION.   The excretion rate of sulfate evidently depends in part upon its serum concentration.   This observation is consistent with that of others (6, 8). The straight lines relating serum concentration and excretion rate do not, however, pass through the origin (figs. 1, 2).   Algebraically the equation of any such lines is of the form

$$(1) \qquad\qquad UV = k(S - a), \quad \text{or} \quad k = \frac{UV}{S - a}$$

where UV is the excretion rate, S the serum concentration, k the slope of the line, and a the distance from the origin to the point of intersection of the abscissa. If this equation were to characterize completely the excretion of sulfate, excretion should cease whenever the concentration in serum declines to the value a. Thus a in each experiment would be an apparent "renal threshold" for sulfate excretion.   Such is, however, not the case.   Even in those experiments in which the value of a approximates that of the endogenous serum sulfate concentration, the equation fails to characterize sulfate excretion properly, since, in point of fact, the excretion of sulfate does continue, though at a much slower rate, at usual endogenous serum concentrations.   Indeed sulfate was long ago classified as a "non-threshold" substance since almost no urine is completely free of sulfate (13).   Even less adequate is the characterization in those experiments (table 1: 6, 7 and 8) in which the extrapolated lines intersect the abscissa well above the usual endogenous concentration.   In fact, a line representing the whole of the experimental data must consist of two parts.   The first, valid at elevated serum concentrations, is represented by equation (1).   At low concentrations this straight line is replaced by another curve having a much less acute slope.   Our data are not adequate to determine whether or not this second portion is exactly a straight line, or whether its junction with the first portion is curved or abrupt. It seems clear, however, that excretion of sulfate is a different function of the serum concentration when it is artificially raised to a high concentration than it is when it lies within the usual range of physiological variation.

Because the lines representing exogenous excretion do not pass through the origin, it is mathematically self-evident that the clearance of sulfate will depend on serum concentration.   This may best be seen by calculating the clearance from equation (1):

$$(2) \qquad\qquad \text{Clearance} = \frac{UV}{S} = k\left(1 - \frac{a}{S}\right)$$

The clearance will evidently be greater the higher the serum concentration.   In this respect the clearance of sulfate differs entirely from those of the organic filterable group, whose representative lines do pass through the origin and whose clearances are therefore independent of serum concentration.   In those instances in which the value of a corresponds to the usual endogenous concentration of sulfate, the excretion of sulfate is simply proportional to the *increase* in concentration of sulfate in serum (equation (1)).   Unfortunately this simple generalization does not hold under all circumstances.

Whole salts rather than individual ions are excreted, since total anion and total cation concentration are always nearly equal. As a necessary consequence of this interdependence of anion and cation the renal excretion rates of both cannot be dependent solely on their respective concentrations in serum. Experiments 7 and 8 of table 1, in which a mixture of sodium sulfate and of sodium chloride was injected, furnish an excellent example of this principle. In the first two periods after injection equal amounts of sodium and of sulfate were excreted in the urine; yet during these same periods the serum concentration of sulfate had varied several fold while that of sodium had changed by only a fraction of its total value. Excretion of sulfate corresponds fairly closely to its concentration in serum, while at the same time the excretion of sodium is dissociated from its serum concentration. Its real determinant appears to be the concentration of sulfate in the urine, enough sodium being excreted to neutralize the sulfate, more or less without regard to its concentration in serum. At least this is the way that the relationship appears. Possibly, if sodium of serum could be increased as much proportionally as is sulfate, this apparent dependence of sodium on sulfate excretion might disappear. This possibility is unfortunately incapable of experimental verification.

There is in fact a linear relationship between excretion rate and serum concentration for sodium as well as for sulfate in these experiments. This is shown in figure 5. The lines following injections of mixtures of sodium chloride and sodium sulfate at first have a very steep slope, then break sharply while the serum concentration is still much elevated. Excretion rate of sodium is thereafter much reduced. Reference to the protocols (table 1) shows the meaning of this. Sodium is excreted very rapidly as sodium sulfate until nearly all the injected sulfate has been eliminated, and then is excreted much more slowly as sodium chloride until the added chloride has been eliminated. In other words, sodium is excreted by different laws depending on the associated anion. A passive rôle of sodium, at least under these circumstances, is again suggested. Although excretion of sodium is functionally related to its concentration in serum, a calculation of sodium clearances alone in these experiments would not clarify the real physiological situation.

The "extra" excretion of sodium after potassium sulfate infusion presents a somewhat more complex problem. This excessive excretion of sodium occurs after ingestion or injection of all potassium salts. Indeed advantage has been taken of this effect in the use of potassium salts as diuretics (9). It is possible that both potassium and sulfate are individually excreted, each according to its own law. Since this would result in an inequality between cation and anion concentration in urine, enough sodium is excreted to preserve electroneutrality. Certainly it is true that, following the injection of various potassium salts, potassium excretion, like sulfate excretion, is closely related to serum potassium concentration and is relatively little affected by the associated anion.

It is not clear whether the increased excretion of potassium after hypertonic sodium sulfate infusions (table 1, expt. 13) represents a similar sort of response, since we have failed to discover any characteristic law of sodium excretion which

would prevent an excretion of sufficient sodium to balance all the sulfate.   This particular effect seems to be one expression of a general type of reaction of the organism to any urgent need for water in the excretion of salts.   For example, increased excretion of potassium may also be produced by the injection or ingestion of hypertonic sodium chloride solutions (2, 3, 4).   A similar excessive renal excretion of potassium may be produced by severe chronic water deprivation, without the injection of any salt (3).   On the other hand, little or no increase in potassium excretion is brought about by the injection of isotonic sodium chloride (15).   The loss of potassium is frequently associated with the development of hypertonicity in the extracellular fluids, but it is not clear that this is an invariable accompaniment.   It may be pointed out that both the development of extracellular hypertonicity and the loss of potassium have one effect in common.   They both release intracellular fluid for extracellular distribution.   The final effect of both reactions is to diminish the degree to which extracellular fluid contracts.   A certain protection to the circulation is thereby provided.   Loss of potassium may, therefore, be not wholly without value to the organism.

In the magnesium sulfate experiments both magnesium and sulfate, though originally provided in equimolecular quantities, and distributing themselves through the same compartment of body fluid, obey their separate laws of excretion consistently in all five experiments.   The characteristic clearances of magnesium are always regularly a little below those of sulfate.   Presumably the excretion of a small amount of some other cation was stimulated, in order to ensure continuous electroneutrality of urine.   Unfortunately no analytical data bearing on this point are available.

The presence of sulfate greatly modifies the excretion of chloride, while the effect of chloride on sulfate excretion is not quantitatively so great.   Indeed the latter is only important in that it establishes the principle that the characteristic mode of excretion of sulfate is modified by other associated anions.   The inhibition of sodium chloride excretion by the simultaneous excretion of sodium sulfate is a much more striking phenomenon, since the usual relation between serum concentration and urinary excretion is exactly reversed.   A great excess of sodium chloride awaits excretion and the serum chloride concentration is already elevated; yet the excretion rate of chloride progressively declines and the concentration of chloride in serum rises still further.   To use an older terminology, chloride slightly decreases the "renal threshold" for sulfate, while sulfate greatly increases the "threshold" for chloride.   This is purely a temporary phenomenon.   As soon as the sulfate has been eliminated the sodium chloride is excreted in its usual fashion.   In effect, the native salt, sodium chloride, is retained until the foreign salt, sodium sulfate, is preferentially almost completely eliminated.   This emphasizes in yet another way the peculiar status of both sodium and of chloride.   Changes in the serum concentrations of both appear to be the results rather than the causes of the changes in their respective urinary excretions.   In these experiments excretion of each of these ions is throughout exactly conditioned to permit

the elimination of the sulfate as rapidly as possible, without regard to their concentrations in serum.

The discrepancy between our results and those of Goudsmit (6) has already been mentioned. He found that at high concentrations of sulfate in serum the sulfate clearance became nearly equal to the creatinine clearance. We, on the other hand, found at similar high serum levels a very irregular relation between creatinine clearance and sulfate clearance (fig. 4). Sometimes they were nearly equal, sometimes not. No consistent tendency to approach one another with rising concentration, such as Goudsmit found, was present. His experiments meant that the rate of tubular reabsorption of sulfate did not keep pace with the greater amount filtered through the glomeruli at high concentrations. In many of our experiments, on the other hand, the rate of reabsorption increased *pari passu* with glomerular filtration. Nowhere was there any evidence of a maximal rate of reabsorption of sulfate, such as was suggested by his experiments. A possible explanation may be found in the way the two groups of experiments were conducted. Goudsmit regularly gave his animals a large amount of saline by stomach tube an hour or so before the experiment, in order to provoke a profuse diuresis. We have already seen in our experiments that administration of sodium chloride along with the sulfate increases the clearance of sulfate and diminishes its tubular reabsorption. It seems entirely likely that a similar effect of sodium chloride was active in Goudsmit's experiments. Certainly in the single experiment of ours (table 1, expt. 12) in which sodium bromide was injected prior to the experiment, the subsequent sulfate clearance closely approached that of creatinine.

The very rough relationship between creatinine and sulfate clearances (fig. 3) indicates that glomerular filtration is only one of several determinants of sulfate clearance. Of course these were all normal animals; were subjects with impaired glomerular filtration included, the relationship might well have been more striking. It has been noted that many of the lines representing sulfate excretion are straight, in spite of markedly varying glomerular filtration. This would seem to mean that, irrespective of the amount delivered to them in the glomerular filtrate, the tubules leave in the urine an amount proportional to the increase in serum concentration $(S - a)$. This relationship may of course well be fortuitous, due to the particular way in which these experiments were conducted.

There is much evidence that the kidney is in some way limited in its ability to produce a hypertonic urine (5, 13). Nothing in these experiments, however, suggests that this apparent osmotic limitation in any way conditioned the excretion of ions. Only moderately hypertonic solutions were used, so that considerable water was always available for excretion. Enough water was taken from the infusion itself and from the body store to keep the concentration of salts in the urine well within the normal range. The highest total osmolar concentration in urine did not usually coincide with the maximal excretion rate of salt immediately after infusion, but appeared later, after the excretion had declined more nearly toward normal. This seems good evidence against total

osmotic limitation of excretion, since if this were the limiting factor one would expect the urines of several periods after infusion to have the same "maximal" total osmolar concentration.

SUMMARY AND CONCLUSIONS

1. The excretion of sulfate and of other anions and cations following intravenous infusions of various sulfates in the dog has been studied.

2. Injected sulfate is excreted according to a different law than endogenous sulfate.

3. At high concentrations of sulfate in serum, sulfate excretion is in part a function of serum concentration, and at times is nearly proportional to the increase in concentration.

4. Sulfate clearance varies systematically with serum concentration, increasing as the serum concentration rises.

5. Sulfate excretion is in the main independent of the associated cation.

6. Sulfate excretion is somewhat increased by the simultaneous injection of sodium chloride.

7. Chloride excretion is almost completely repressed by sulfate excretion, even when a large sodium chloride infusion is given simultaneously with the sulfate infusion.

8. The extent to which ions may be excreted according to their own characteristic laws and the means by which electroneutrality of urine is maintained are discussed in the light of these experiments.

REFERENCES

(1) COPE, C. L. Biochem. J. 25: 1183, 1931.
(2) DARROW, D. C. AND H. YANNET. J. Clin. Investigation 15: 419, 1936.
(3) ELKINTON, J. R., JR. AND M. TAFFEL. J. Clin. Investigation: in press.
(4) GAMBLE, J. Chemical anatomy, physiology and pathology of extracellular fluid. Chart 34. Department of Pediatrics, Harvard Medical School, Boston. 3rd ed., 1941.
(5) GILMAN, A. AND N. E. KIDD. This Journal 123: P77, 1938.
(6) GOUDSMIT, A., JR., M. H. POWER AND J. L. BOLLMAN. This Journal 125: 506, 1939.
(7) HALD, P. M. J. Biol. Chem. 103: 471, 1933.
(8) HAYMAN, J. M., JR. AND S. M. JOHNSTON. J. Clin. Investigation 11: 607, 1932.
(9) KEITH, N. M. AND M. W. BINGER. J. A. M. A. 105: 1584, 1935.
(10) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Volume II, Methods. Baltimore, 1932.
(11) SHANNON, J. A. This Journal 112: 405, 1935.
(12) SHANNON, J. A. This Journal 114: 362, 1936.
(13) SMITH, H. W. The physiology of the kidney. New York, 1937.
(14) SMITH, P. K., A. W. WINKLER AND B. M. SCHWARTZ. J. Biol. Chem. 129: 51, 1939.
(15) STEWART, J. D. AND G. M. ROURKE. J. Clin. Investigation 21: 197, 1942.
(16) WINKLER, A. W. AND J. PARRA. J. Clin. Investigation 16: 859, 1937.

THE RÔLE OF THE ANTERIOR PITUITARY IN THE MAINTENANCE OF NORMAL BLOOD SUGAR LEVELS AND IN THE PHYSIOLOGICAL MOBILIZATION OF LIVER GLYCOGEN[1]

R. C. DE BODO, H. I. BLOCH AND I. SLATER

From the Department of Pharmacology, New York University College of Medicine

In an earlier paper it has been shown that the intravenous infusion of adrenaline produces only a very slight rise in blood sugar in hypophysectomized dogs in contrast to the marked hyperglycemia it produces in normals. This phenomenon cannot be explained on the basis of inadequate liver glycogen stores, since some of our hypophysectomized dogs had liver glycogen stores within normal limits, others had smaller stores, but all had amounts of liver glycogen which, if present in normal dogs, would have been sufficient to have produced a marked hyperglycemia (de Bodo, Bloch and Gross, 1942). On the basis of these earlier experiments it was concluded that in the absence of the anterior pituitary there is an impairment in the mobilization of liver glycogen by infused adrenaline.

With this conclusion in mind the question arose as to whether the liver glycogen of hypophysectomized dogs is also resistant 1, to adrenaline secreted under physiological conditions, and 2, to other glycogenolytic agents which may function under physiological conditions. If mobilization in response to these agents is also impaired by hypophysectomy, then low post-absorptive blood sugar values should be found coexisting with adequate liver glycogen stores in hypophysectomized dogs.

The literature dealing with the post-absorptive blood sugar levels of hypophysectomized animals is contradictory. Some investigators have reported values lower than those found in normal animals, others have found no change in post-absorptive blood sugar levels after hypophysectomy. This literature is thoroughly reviewed by Houssay (1935) and by Van Dyke (1936, 1939). All investigators are agreed that hypophysectomized animals develop hypoglycemia after fasting, and have a tendency to go spontaneously from an apparently normal state into hypoglycemic shock. However, all these workers have made only random observations of the post-absorptive blood sugar levels of hypophysectomized animals, but have not followed them daily from the time of the hypophysectomy. In view of these facts we have made a systematic study of the effect of hypophysectomy on the post-absorptive blood sugar levels and on the liver glycogen contents of a series of dogs.

METHODS. Dogs were used exclusively in this study. Hypophysectomy was performed by the oral approach (except when otherwise specified) and the pituitary gland removed in one piece, which included the pars distalis, pars

intermedia, pars nervosa, and pars tuberalis. (For further details see de Bodo et al., 1942.) The animals were maintained on a constant diet for two weeks prior to hypophysectomy and throughout the period of observation following hypophysectomy. In every case it was ascertained that the allotted ration was consumed and retained.

Post-absorptive blood sugar levels (17 hrs. after the last feeding) were determined prior to hypophysectomy and almost daily thereafter (except when otherwise specified). In addition, in certain instances blood sugar samples were drawn 24 hours after the last feeding. Immediately prior to the termination of each experiment by the sacrifice of the animal, liver samples for glycogen determination were excised under local anesthesia. The blood samples were drawn without the use of any anesthesia. Blood sugar determinations were made by the Hagedorn-Jensen method (1923), using the Somogyi (1930) acid-zinc filtrate. Liver glycogen determinations were made by a modified Pflüger method (Bodo and Neuwirth, 1933).

At autopsy the organs were fixed in formalin and after 24 hours in the fixative the weights of the endocrine glands were determined, with special attention to the adrenals, pancreases, and gonads. Histological studies were made of sections of a block including the body of the sphenoid bone, the fibrous tissue occupying the sella turcica, and the overlying brain tissue in each animal. In addition histological studies were made of the organs of each animal, with special attention to the thyroids, adrenals, gonads, and pancreas, as well as to the removed pituitary gland. These histological studies were made by Dr. David Marine, Director of the Laboratories of Montefiore Hospital, New York City.[2]

RESULTS. In figures 1 to 7 we have presented graphically the day-to-day variations observed in the post-absorptive blood sugar levels of seven hypophy-

Figs. 1–7. Blood sugar changes in 7 dogs during the period following hypophysectomy, with liver glycogen values found at the termination of each experiment. The numbers on the abscissae represent days after hypophysectomy. The numbers on the ordinates to the left of each curve represent blood sugar in milligrams per cent. The numbers on the ordinates to the right of each curve represent the liver glycogen in per cent. The encircled dots represent post-absorptive (17 hrs. after last meal) blood sugar values before hypophysectomy. The simple dots represent post-absorptive (17 hrs. after last meal) blood sugar values after hypophysectomy. The solid triangles represent blood sugar values 24 hours after last meal. The solid vertical columns represent liver glycogen values. The hatched' areas represent range of post-absorptive (17 hrs. after last meal) blood sugar concentration of 18 normal dogs maintained for a period of weeks on the same diet as the hypophysectomized dogs.

Fig. 8. Fluctuations in post-absorptive blood sugar values of 6 normal dogs during a period of three weeks. The numbers on the abscissa represent days of the experiment. The numbers on the ordinate represent blood sugar in milligrams per cent. Each of the six symbols used in this figure represents the post-absorptive (17 hrs. after last meal) blood sugar values of one normal dog. Note that the fluctuations in the values for each dog do not exceed 10 mgm. per cent.

FIG. 1
DOG H 7

FIG. 2
DOG H 9

FIG. 3
DOG H 10

FIG. 4
DOG H 11

FIG. 5
DOG H 12

FIG. 6
DOG H 13

FIG. 7
DOG H 14

FIG. 8

Figs. 1-8
673
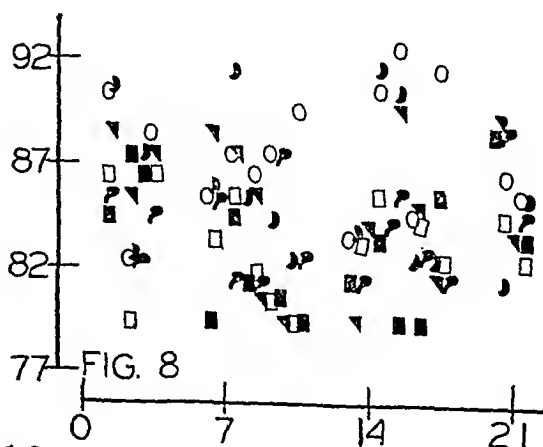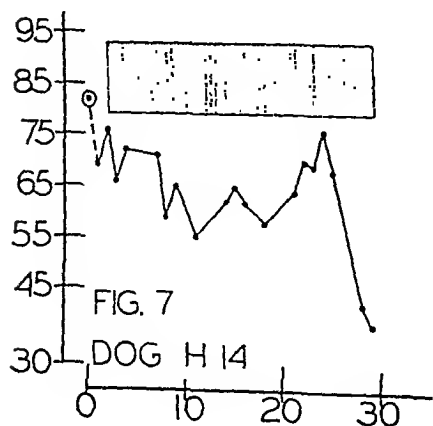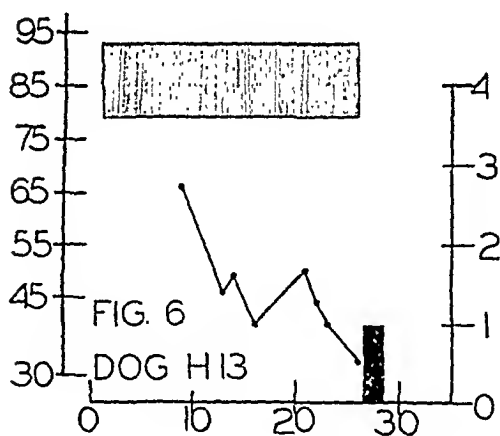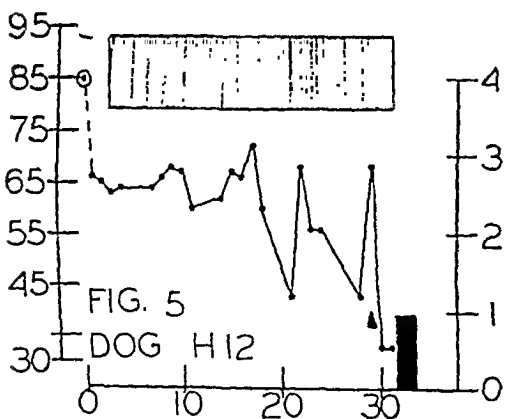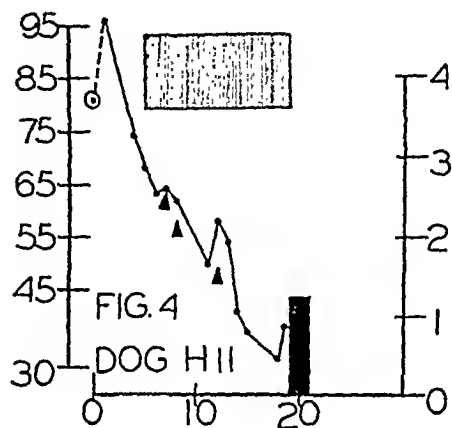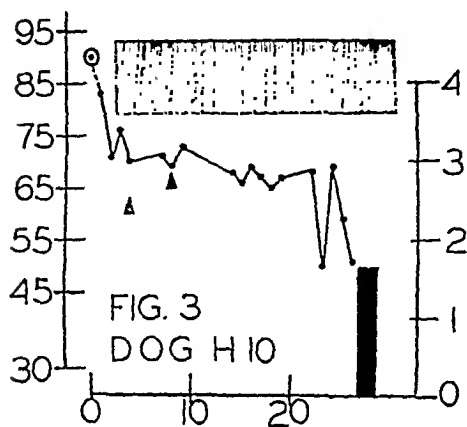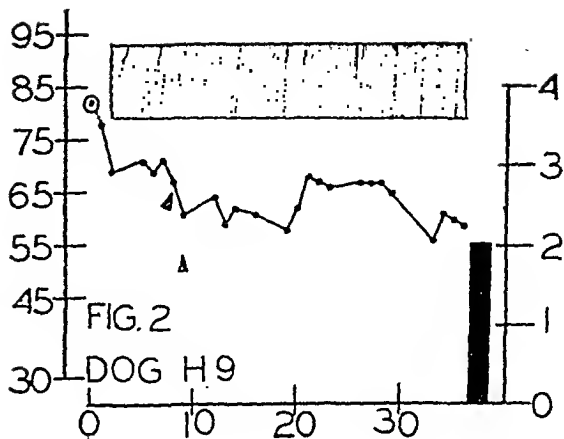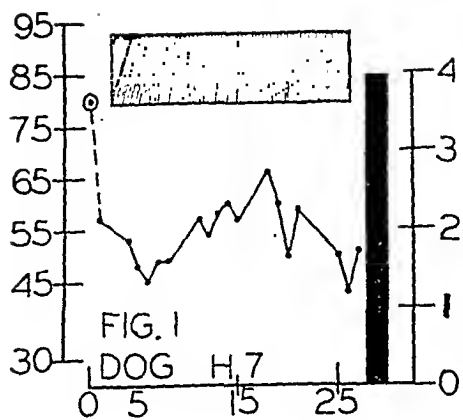
sectomized dogs. It can be seen that in the first two post-operative days a sharp decrease in the post-absorptive blood sugar value is apparent, except in the case of dog H11. This dog was the only one of those observed which had a higher post-absorptive blood sugar concentration the day after hypophysectomy. However, it should be noted that shortly thereafter the blood sugar concentration fell to below its preoperative level and continued to fall from then on.

During the weeks of observation following the hypophysectomy the post-absorptive blood sugar concentrations of each of these seven dogs fluctuated from day to day but were always at levels substantially lower than those maintained before hypophysectomy. The hatched area in each figure represents the range of post-absorptive blood sugar concentration (79–93 mgm. per cent) of 18 normal dogs maintained for a period of weeks on the same constant diet as that fed to the hypophysectomized dogs.

A glance at the seven curves (figs. 1–7) reveals the striking range of the fluctuations in post-absorptive blood sugar values in the hypophysectomized dogs during the period of observation. In figure 8 the post-absorptive blood sugar concentrations of 6 normal dogs studied for a period of three weeks are given. As can be seen the fluctuations in the post-absorptive blood sugar values of each individual dog did not exceed 10 mgm. per cent, whereas among the hypophysectomized dogs the most limited range seen was that of dog H9 (56–71 mgm. per cent = 15 mgm. per cent, even disregarding the blood sugar value of ·78 mgm. per cent on the first post-operative day). In the other hypophysectomized dogs the ranges in milligrams per cent were 26, 23, 39, 38, 42 and 33.

The experiments on dogs H11, H12, and H13 were terminated when their blood sugar concentrations fell to 33 to 38 mgm. per cent, at which time these dogs refused to eat voluntarily and vomited the food ·fed by stomach tube. With blood sugar values of 32 to 33 mgm. per cent H11 was unable to walk, H12 showed twitches, and H13 was ataxic. None of these dogs showed actual convulsions or coma. No attempt was made to raise the blood sugar level of these dogs either by sugar infusion or by the administration of adrenal cortical extract. Liver samples were taken, under local anesthesia, for glycogen determinations, and the animals were then sacrificed. The experiment on dog H14 was terminated by the death of the animal. The blood sugar concentration fell to 38 mgm. per cent, the animal was ataxic, vomited the food fed by stomach tube, and was found dead the following morning. Thus, due to the autolysis that set in, it was impossible to obtain valid liver glycogen values or to make adequate histological studies of the sella turcica or organs of this animal.

The experiments on dogs H7, H9 and H10 were terminated while their post-absorptive blood sugar concentrations, although markedly lower than those found in normal animals, were still considerably higher than those found in dogs H11, H12, H13, and H14 at the time they were sacrificed. The symptoms observed in these latter dogs when their blood sugar concentrations fell to 33 to 38 mgm. per cent were never seen in dogs H7, H9, and H10.

Dog H11 showed the most abrupt and steady fall in post-absorptive blood

sugar level in the weeks following hypophysectomy. Paradoxically, this is the only animal of those presented here which had in one section of the sella turcica some blurred cells which might have been anterior pituitary cells. Also the interstitial cells of the gonads of this dog showed some signs of activity, and the adrenal cortices were not as atrophic as those of some of the other animals. In all the other dogs histological examination established the complete absence of all pituitary tissue.

All these dogs had very small adrenal glands which showed the cortical atrophy characteristic of total hypophysectomy (de Bodo and Marine), although the degree to which the atrophic changes had progressed varied somewhat from animal to animal. No correlation could be detected between the rapidity and constancy with which the post-absorptive blood sugar level fell and the degree of adrenal cortical atrophy. For example, dog H10 showed a much greater atrophy than did dog H13 or dog H11, although its blood sugar concentration never fell as low.

TABLE 1

Simultaneously determined blood sugar and liver glycogen values of hypophysectomized dogs

DOG NUMBER	BLOOD SUGAR*	LIVER GLYCOGEN	DOG NUMBER	BLOOD SUGAR*	LIVER GLYCOGEN
	mgm %	*G.%*		*mgm.%*	*G.%*
H5	54	4.42	H16	34	2.04
H6	54	4.02	H10	51	1.65
H7	52	3.95	H11	38	1.22
H8	53	2.86	H12	33	0.94
H15	45	2.86	H13	33	0.98
H9	59	2.05			

* Postabsorptive value.

Of the four dogs which showed the extremely low blood sugar concentrations of 33 to 38 mgm. per cent, three (H12, H13, H14) had been given insulin (0.025 U/kilo intravenously) on two previous occasions. Dog H11 had never been given insulin. The other three of the hypophysectomized dogs studied (H7, H9, H10) were never given insulin. On the basis of this and many other experiments on hypophysectomized dogs not included here, we believe that insulin administration may be one factor which increases the tendency to "spontaneous" hypoglycemic crises in the hypophysectomized animal, even though the animal recovers from the immediate effects of the drug after the intravenous administration of sugar. In other words, though the dog may be brought out of the insulin coma by intravenous glucose and may then appear well, resume activity and eat voluntarily, still a few days after insulin the animal shows a more pronounced tendency to hypoglycemic crises than do hypophysectomized dogs which have never been given insulin.

The amounts of glycogen contained in the livers of these hypophysectomized dogs at the time when the last blood sample was drawn for glucose determination are shown in figures 1 to 6 and in table 1. We have also included in table 1

the results obtained on 5 dogs, hypophysectomized by the temporal approach, whose blood sugar values were not determined daily. Their blood sugar was determined on several occasions, including of course a determination made immediately prior to the excision of the liver samples for glycogen determination. These animals were not 100 per cent total hypophysectomies, in that · the histological examination revealed small remnants of anterior pituitary tissue in their sellae turcicae. However, since these hypophyseal remnants were not functionally adequate to prevent the atrophic changes in the adrenal cortices, thyroids, and gonads characteristic of our totally hypophysectomized animals, and since these dogs reacted to insulin and to fasting exactly as did the histologically 100 per cent hypophysectomized animals, we regard these dogs as functionally hypophysectomized.

Considering the figures in table 1 it can be seen that in hypophysectomized dogs a liver glycogen concentration of approximately 4 per cent can coexist with a post-absorptive blood sugar of 52 to 54 mgm. per cent (dogs H5, H6, H7). This amount of liver glycogen is within the normal range, but in a normal dog it would never coexist with such a low blood sugar concentration. Normal dogs with liver glycogen stores in this range have blood sugar values of 79 to 93 mgm. per cent (average 84 mgm. per cent) (de Bodo et al., 1942), about 30 mgm. per cent greater than those found in these hypophysectomized dogs. Not every hypophysectomized dog with post-absorptive blood sugar values of approximately 50 mgm. per cent had as much as 4 per cent liver glycogen. As can be seen in table 1, some (dogs H8, H15, H9, H10) had less liver glycogen.

In dogs H11, H12, and H13 the extremely low blood sugar concentration of 33 to 38 mgm. per cent coexisted with a liver glycogen concentration of approximately 1 per cent. Liver glycogen values of this order may be found in normal dogs only after a period of 8 to 10 days of fasting, but when found they coexist with blood sugar concentrations of 58 to 76 mgm. per cent (average 65 mgm. per cent) (de Bodo et al., 1942), again about 30 mgm. per cent greater than that found in dogs H11, H12, and H13. In dog H16 a blood sugar value of 34 mgm. per cent coexisted with a liver glycogen content of 2.04 per cent. This relatively large amount of liver glycogen with such an extremely low post-absorptive blood sugar value was an unusual finding in our series. In short, the liver glycogen values found in the hypophysectomized dogs listed in table 1 vary widely, but it should be noted that in every instance the coexisting blood sugar concentrations were markedly lower in these animals than they would have been in normal animals with the corresponding liver glycogen values.

In addition to the determination of the daily post-absorptive blood sugar values, the 24 hour fasting blood sugar level was also determined in dogs H9, H10, H11 and H12. These results are shown in figures 2, 3, 4 and 5. It can be noted that, as would be expected, these values are all lower than the corresponding post-absorptive values. However, of all the figures obtained only two (dog H11, 12 days post-operatively and dog H12, 29 days post-operatively) were lower than 50 mgm. per cent. Soskin et al. (1935–6, 1938, 1939) used the 24 hour fasting blood sugar level as a criterion for the completeness of their

hypophysectomies, accepting as completely hypophysectomized only those animals with blood sugars below 50 mgm. per cent. If we were to accept this we would have to regard as incompletely hypophysectomized dogs H9 and H10, each of which had on two occasions 24 hour fasting values over 50 mgm. per cent (65, 53; 63, 68 mgm. per cent), and which were subsequently proved by histological study to be 100 per cent completely hypophysectomized.

CONCLUSIONS AND DISCUSSION. From the results presented here it is apparent that within two days following hypophysectomy the post-absorptive blood sugar of dogs falls to a level considerably below its pre-operative value to which it never returns. During the weeks subsequent to hypophysectomy the post-absorptive blood sugar fluctuates widely around the lower level with a tendency to fall further rather than to rise. The blood sugar never falls from normal to hypoglycemic shock levels without passing gradually (with or without marked fluctuations) through successively lower stages. Day-to-day observation of the post-absorptive blood sugar level of hypophysectomized dogs makes it possible to detect impending hypoglycemic crisis.

Since the animals on which these observations were made were found histologically to be 100 per cent completely hypophysectomized and since all had the adrenal cortical atrophy which is as characteristic of the hypophysectomized dog (de Bodo and Marine) as it is of the hypophysectomized rat (Smith, 1930), we believe that the inability to maintain normal blood sugar levels is also a characteristic of complete hypophysectomy.

Houssay (1935) does not consider subnormal blood sugar values characteristic of hypophysectomy in the dog. In one of their papers Houssay and Biasotti (1931a) reported blood sugar levels below normal in only 2 out of 5 of their hypophysectomized dogs. However, it should be noted in this connection that Houssay (1936) has stated that adrenal cortical atrophy occurs "less frequently" in hypophysectomized dogs than in hypophysectomized rats. In a paper in which Houssay and Biasotti (1931b) presented histological data, only 1 of the 5 hypophysectomized animals studied showed marked adrenal cortical atrophy, 1 showed moderate atrophy, and the remaining 3 had normal adrenal cortices. In a later paper Houssay (1933) charted the weights of the adrenal glands of 20 of his hypophysectomized dogs, on which no blood sugar findings were presented. We have related the weights of the adrenal glands as given in Houssay's chart to the body weights of his animals. This reveals that only 7 out of his 20 hypophysectomized animals showed a degree of adrenal atrophy comparable to that which we (de Bodo and Marine) observed in all our 100 per cent completely hypophysectomized animals.

Chaikoff et al. (1935) found low post-absorptive blood sugar values in only 2 out of 7 hypophysectomized animals studied. Unfortunately no statements are made as to the condition of the adrenal cortices of these animals.

On the other hand, our results are in agreement with those of Smith et al. (1936) who, although they did not follow blood sugar levels daily before and after hypophysectomy, found that the post-absorptive blood sugar level of hypophysectomized monkeys (33 observations on 9 animals) varies around an

average value of 59 mgm. per cent as contrasted with an average of 110 mgm. per cent found in normal monkeys (53 observations on 24 animals).

The characteristically low post-absorptive blood sugar level of hypophysectomized dogs is found in the presence of fairly large amounts of liver glycogen. The co-existence of these two findings requires further comment. The blood sugar level is determined by the ratio of glucose production to glucose utilization. There is no general agreement as to the effects of hypophysectomy on glucose utilization. Whereas Chambers et al. (1935) were unable to detect any change, and Soskin et al. (1938, 1939) found it decreased, Fischer, Russell and Cori (1936) and Russell (1942a, 1942b) found increased glucose utilization after hypophysectomy in the rat, as did Greeley (1940) in the rabbit.

Assuming that the latter view is correct and that glucose utilization is increased after hypophysectomy, this fact alone could not account for the low blood sugar levels found in our hypophysectomized animals in the presence of the amounts of glycogen contained in their livers. Russell (1942a) claimed that glucose, utilization is *twice* as great after hypophysectomy in the rat. As has been shown by Bodo, Barker and Benaglia (1938), normal dogs in the post-absorptive state maintained normal blood sugar levels while performing moderate muscular exercise, during which their glucose utilization was proved to be *four times* increased as determined by the respiratory metabolism. It is clear then that the production of glucose in the normal animal is capable of keeping pace with even a fourfold increase in utilization.

Therefore it is obvious that the low post-absorptive blood sugar values in our hypophysectomized animals cannot be accounted for *solely* on the basis of increased utilization. There must be decreased production of sugar. There is ample evidence that the production of sugar from non-carbohydrate sources is deficient after hypophysectomy, but since our animals had liver glycogen reserves (in some cases within normal limits) the defective formation of sugar from non-carbohydrate sources cannot have been the *only* limitation on glucose production. We must therefore conclude that there is *also* a defective glucose formation from liver glycogen. In other words, after hypophysectomy there is an impairment in the physiological mobilization of liver glycogen.

There is convincing evidence available that when the blood sugar of a normal animal is markedly lowered (to a "critical level") by large doses of insulin, adrenaline is secreted—as demonstrated on the animal's sensitized denervated heart—which in turn mobilizes the liver glycogen (Cannon et al., 1924). It is questionable whether adrenaline secretion occurs when the blood sugar is not lowered as drastically but only to such a slight degree as occurs under truly physiological conditions, as in the course of moderate muscular exercise. In the paper referred to above (Bodo et al., 1938) it was demonstrated that during moderate muscular exercise such liver glycogen mobilization as is necessary to maintain normal blood sugar levels occurs not only in normal but also in adrenal-inactivated (right adrenal removed, left denervated and demedullated) and liver-denervated animals. Therefore liver glycogen mobilization can be effected in non-hypophysectomized dogs by agents other than adrenaline and liver nerves.

In our hypophysectomized animals the blood sugar levels were so low that it is probable that not only were these agents called out but also adrenaline must have been secreted. Cope and Marks (1934–1935) have shown that hypophysectomy does not prevent the secretion of adrenaline in response to insulin hypoglycemia. Our own histological studies have shown that the adrenal medullae after hypophysectomy do not become atrophic but rather hypertrophic. The cells of the medullae are large, columnar, suggestive of great secretory activity (de Bodo and Marine). Since in these hypophysectomized dogs of ours the low blood sugar values coexisted with adequate liver glycogen stores it is apparent that neither secreted adrenaline nor the other physiological agents which are capable of mobilizing liver glycogen in the non-hypophysectomized animal are effective in the absence of the anterior pituitary. This impaired mobilization of liver glycogen must be one of the factors responsible for the characteristically low post-absorptive blood sugar level of hypophysectomized dogs.

SUMMARY

1. Within two days following hypophysectomy the post-absorptive blood sugar of dogs falls to a level considerably below its pre-operative value to which it never returns.

2. During the weeks subsequent to hypophysectomy the post-absorptive blood sugar fluctuates widely around the lower level with a tendency to fall further rather than to rise.

3. With these markedly low post-absorptive blood sugar levels some of the hypophysectomized dogs had liver glycogen stores within normal limits, others had smaller stores, but all had amounts of liver glycogen which, if present in non-hypophysectomized dogs, would have been sufficient to have maintained normal blood sugar levels.

4. In the absence of the anterior pituitary there is an impairment in the mobilization of liver glycogen by secreted adrenaline and also by the other physiological agents which are capable of mobilizing it in the non-hypophysectomized animal.

5. In the absence of the anterior pituitary there is an inability to maintain normal blood sugar levels.

6. The impairment in the physiological mobilization of liver glycogen is one of the factors responsible for the characteristically low post-absorptive blood sugar level of hypophysectomized dogs.

REFERENCES

Bodo, R. C., S. B. Barker and A. E. Benaglia. This Journal 123: 18, 1938.
de Bodo, R. C., H. I. Bloch and I. H. Gross. This Journal 137: 124, 1942.
de Bodo, R. C. and D. Marine. In preparation.
Bodo, R. C. and I. Neuwirth. This Journal 87: 1, 1933.
Cannon, W. B., M. A. McIver and S. W. Bliss. This Journal 69: 46, 1924.
Chaikoff, I. L., F. L. Reichert, P. S. Larson and M. E. Mathes. This Journal 112: 493, 1935.
Chambers, W. H., J. E. Sweet and J. P. Chandler. This Journal 113: 26, 1935.
Cope, O. and H. P. Marks. J. Physiol. 83: 157, 1934–35.

FISHER, R. E., J. A. RUSSELL AND C. F. CORI.   J. Biol. Chem. **115**: 627, 1936.

GREELY, P. O.   Endocrinology **27**: 317, 1940.

HAGEDORN, H. C. AND B. N. JENSEN.   Biochem. Ztschr. **135**: 46, 1923.

HOUSSAY, B. A.   Prensa méd. argent. **20²**: 1563, 1933.
>   Functions of the pituitary gland.   Dunham Lecture no. 3.   Nov. 1935.
>   New England J. Med. **214**: 1023, 1936.

HOUSSAY, B. A. AND A. BIASOTTI.   Pflüger's Arch. **227**: 657, 1931a.
>   Pflüger's Arch. **227**: 664, 1931b.

RUSSELL, J. A.   This Journal **136**: 95, 1942a.
>   Federation Proc. Part II, **1**: 76, 1942b.

SMITH, P. E.   Am. J. Anat. **45**: 205, 1930.

SMITH, P. E., L. DOTTI, H. H. TYNDALE AND E. T. ENGLE.   Proc. Soc. Exper. Biol. and
>   Med. **34**: 247, 1936.

SOMOGYI, M.   J. Biol. Chem. **86**: 655, 1930.

SOSKIN, S., R. LEVINE AND R. E. HELLER.   Proc. Soc. Exper. Biol. and Med. **38**: 6, 1938.

SOSKIN, S., R. LEVINE AND W. LEHMANN.   This Journal **127**: 463, 1939.

SOSKIN, S., I. A. MIRSKY, L. M. ZIMMERMAN AND N. CROHN.   This Journal **114**: 110, 1935–36.

VAN DYKE, H. B.   The physiology and pharmacology of the pituitary body.   Univ. of
>   Chicago Press, Chicago, Ill.   Vol. 1, 1936.   Vol. II, 1939.

THE CONTROL OF CLONIC RESPONSES OF THE CEREBRAL CORTEX

A. ROSENBLUETH, D. D. BOND[1] AND W. B. CANNON

From the Department of Physiology in the Harvard Medical School

In a previous communication (Rosenblueth and Cannon, 1942) a study was made of some features of the tonic-clonic, selfsustained responses of the cerebral cortex to electric stimulation. The responses were found similar in different cortical areas. When activity spread to several areas a physiological coupling was revealed by the temporal correlation of their clonic bursts and usually by the simultaneity of the sudden end of the response at all the regions involved.

The present study attempts an analysis of the mechanism by which different cortical regions may become coupled during clonic activity, and also an elucidation of the factors which determine the rate of clonus, and the reason for the simultaneous abrupt end of the responses in several areas.

METHOD. Young Rhesus monkeys were used, anesthetized with chloralose (0.06 to 0.1 gram per kgm., intravenously). The left cerebral hemisphere was largely exposed, and the right cranium also was opened to permit the approach to the arm region of the motor and sensory areas on that side.

Two light brass stands were firmly screwed into the remaining bone. To these stands were attached 6 pairs of silver electrodes which were placed on various cortical areas and were used for either stimulating or recording. Even marked movements of the head of the animals did not disturb the contact of the electrodes with the brain.

Repetitive stimuli capable of producing tonic-clonic responses were obtained from a Harvard induction coil with a 1.5 v. cell in the primary. The stimulating electrodes were applied by hand. The single-shock stimuli were condenser discharges with a time constant of approximately 0.8 msec. and with a strength of 5 to 30 v. Frequencies of 0.5 to 4 per sec. were used, regulated by changing the bias of a thyratron with a potentiometer. The single shocks were delivered through one of the fixed pairs of electrodes on the cortex.

Both for stimulation and for recording, the interelectrodal distances were from 2 to 5 mm.

The cortical electric responses were recorded by means of a Grass 6-channel set of ink-writing, moving-coil galvanometers and associated resistance-capacity coupled amplifiers. The input was on push-pull for each of the recording pairs of electrodes. The animal was grounded through a diffuse lead attached to either temporal muscle. The records in these conditions are quite independent.

RESULTS. A. *The control of clonic activity by single shocks applied to the cortex.* Rosenblueth and Cannon (1942) showed that stimulation of the cortex by single shocks in the course or shortly after the end of a tonic-clonic sequence could elicit

[1] Fellow of the Rockefeller Foundation.

clonic-like responses. This observation led to the expectation that the rate of clonus could be controlled, and clonic activity could be prolonged by means of trains of shocks with appropriate frequencies. The expectation was confirmed, as follows.

Series of clonic discharges were observed in 3 different conditions: *a*, during a selfsustained response which was not modified by additional single-shock stimulation—the term "selfsustained" will be used to denote this clonic activity, emphasized, when convenient, by the adjective "undriven"; *b*, in the course of a selfsustained response the rate of a clonus could be controlled by appropriate low frequency stimuli—these bursts will be referred to as "driven or controlled" selfsustained clonus; *c*, a clonic response could be prolonged beyond its inherent duration by appropriate single-shock stimulation—this clonus will be designated by the term "prolonged." In order to decide whether single shocks were controlling a selfsustained clonic response the frequency of the shocks was usually varied in the course of the observation. When the clonus followed these variable rates a positive correlation was inferred—i.e., a non-fortuitous coincidence. The prolongation of a clonic series was evidenced not only by the duration, but also by the immediate cessation of the responses upon discontinuance of the stimuli.

For single-shock stimulation to be effective in the control and prolongation of a clonic response several conditions had to be satisfied. *a*. The stimuli had to be stronger than a given critical voltage. It is obvious, therefore, that the threshold of certain cortical elements should be attained. *b*. An appropriate area of the cortex had to be stimulated. This condition is detailed in section B. *c*. It was necessary that the rate of the shocks be adequate, as follows.

A shock applied shortly (within about 0.3 sec.) after a spontaneous clonic burst had occurred, failed to activate the cortex. Hence, when single shocks were repeated faster than about 3.5 per sec. the clonic bursts were never seen to follow each stimulus. The elements of the cortex involved in a clonic response behave, therefore, as if they have a prolonged functional refractory period of about 0.3 sec. This imposes an upper limit on the rate of the effective driving shocks.

Although the maximal rate of clonus which could be imposed at any of the areas tested was about 3.5 per sec., this rate was attainable only early during the clonic response to a prolonged, intense, rapid stimulation. If the stimuli which elicited a tonic-clonic response were weak, or were applied for a brief period, or if a tonic-clonic response had already proceeded for some time, then the imposed rate of clonus could usually follow only lower frequencies. Whenever the rate of driving stimulation was higher than the maximal rate to which the cortex could respond at the time, clonic bursts were elicited by alternate shocks. Responses to every 2nd, or even to every 3rd shock were therefore often seen even though the frequency of stimulation was only 3 per sec. or less.

In figure 1A is illustrated a typical 1:2 alternation. All the active areas share alike in this alternation; this was usual. Figure 1B illustrates an exceptional instance of alternation; the following cycles repeat regularly: short latency → long latency → no response.

If in the course of a clonic response single-shocks were applied with a frequency

slower than that of the prevailing intrinsic rhythm, they did not gain control of the rate. Even if one of the shocks elicited a burst, the next clonic complex occurred before the following shock, and there was no correlation between the stimuli and the clonus.

The rate of stimulation, for prolongation of a clonic response, could not be slower than about 0.7 per sec. Intervals greater than 1.5 sec. invariably resulted
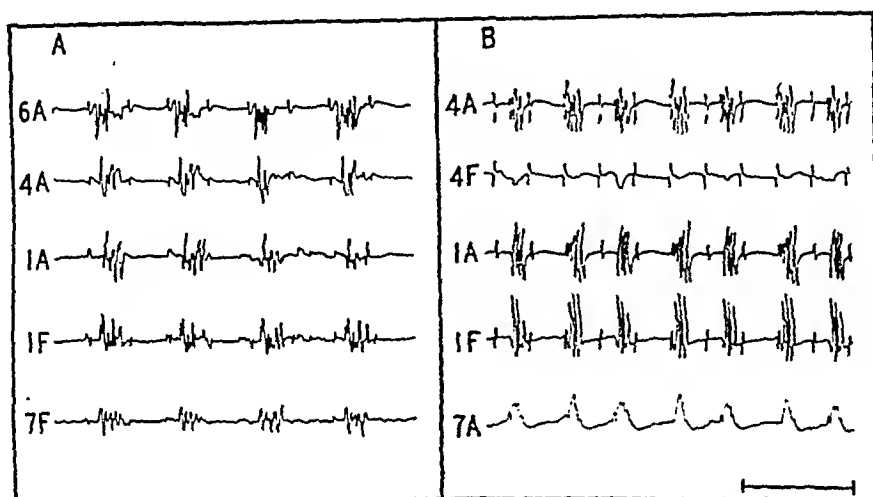
Fig. 1. Alternation of clonic responses to single shocks applied to the cortex. In these and the following figures the records show the electric activity of various cortical areas, unless otherwise stated. The order in which the recording areas are listed corresponds to the successive tracings from above downward. The numbers correspond to Brodmann's (1905) classification. The letters F, A and L, when they follow a number, indicate that the record is from the face, arm or leg division of that area. The letters R or L, when they precede a number, indicate the right or left hemisphere; when there is no preceding letter the area was in the left hemisphere. The speed of the tracings is indicated by the 1-sec. time calibration at the right lower corner. The records all begin some time after a brief (2 to 5 sec.) period of faradic stimulation at tetanic frequency (referred to as rapid stimulation) had initiated a tonic-clonic response in the cortex.

A. Records: 6A, 4A, 1A, 1F, and 7F. Previous rapid stimulation of 6A. Single shocks were applied to 8, as indicated by the artifacts, particularly recognizable in 6A. The clonic bursts occur in response to every other shock.

B. Records: 4A, 4F, 1A, 1F, and 7A. Previous rapid stimulation of 4A. Single shocks were applied to 6, as indicated by the artifacts in 4F. The responses follow the stimuli according to the repeated cycle: short latency → long latency → no response. This unusual mode of alternation endured for 30 sec. and was then followed by one of the habitual modes.

in failure of the clonic responses to follow later stimuli. There is thus a lower limiting frequency of stimulation below which clonic bursts cannot be sustained.

It is interesting that the two limiting frequencies mentioned (3.5 and 0.7 per sec.) coincide approximately with the limits of clonic frequency in selfsustained, undriven responses (3 and 1 per sec.; Rosenblueth and Cannon, 1942).

The degree of prolongation of a clonic response by single-shock stimulation depended on the rate of the stimuli. The results were not accurate enough for a precise quantitative statement, but as a rule a frequency of about 3 per sec. pro-

longed the response more than did a rate of 1 per sec.   In no instance could the discharges be prolonged indefinitely—i.e., beyond about 5 min.   The prolongation depended not only on the rate of the single stimuli but also on the degree of rapid stimulation applied to originate the tonic-clonic response.   The same fre-
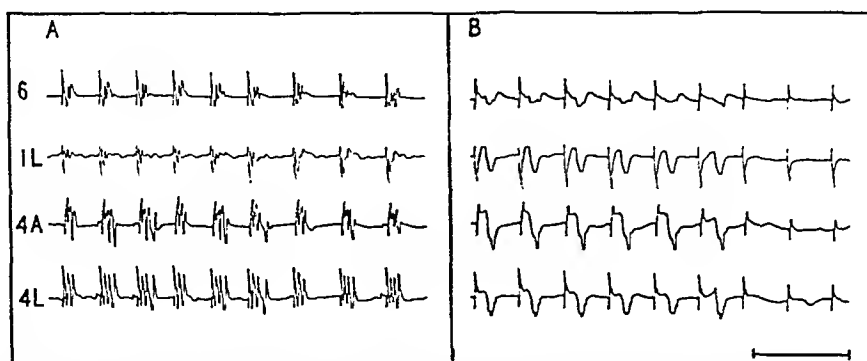
Fig. 2. One mode of ending of driven clonic responses. Records: 6, 1L, 4A, and 4L. Rapid stimulation of 4A initiated tonic-clonic activity which spread to all of the recording regions. Single shocks were applied to 8 at the frequency shown by the stimulus artifacts (see end of B). These shocks controlled the clonic discharges. A illustrates typical responses early in the series. In B (40 sec. later) the responses have lost the spike components.
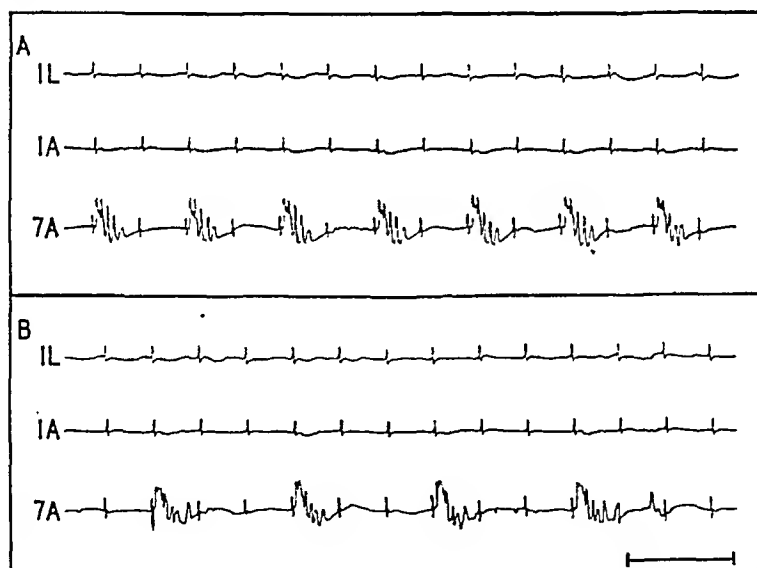
Fig. 3.   Another mode of ending of driven clonic responses. Records: 1L, 1A and 7A. Localized tonic-clonic activity was initiated in 7. The stimulus artifacts denote single-shock stimulation of 6. In A the clonic bursts bear a 1:2 ratio with the stimuli. In B, 10 sec. later, the ratio is 1:3. The last burst in the record was the last clonic response evoked.

quency of the prolonging stimuli was more effective if strong and long initial rapid stimulation had been applied, thus causing a widespread tonic-clonic response, than it was if weak and brief initial stimulation caused only localized selfsustained activity.

The end of clonic activity in response to single shocks of an appropriate fre-

quency could take place in one of several manners. First, with regard to rate, the clonic bursts could follow each single shock to a sudden abrupt end of the response (fig. 2), or else, after having followed a 1:1 relation with the stimuli for some time, they could begin to alternate so that they bore a 1:2, or, later, a 1:3 relation with the shocks, until they finally stopped (fig. 3).

Second, the latency of the clonic bursts elicited by the single shocks was sometimes constant until the responses disappeared (fig. 3). Sometimes, however, toward the end of a series a gradual increase of latency was seen (fig. 2).

Third, with regard to phase or pattern, the clonic responses sometimes remained quite constant until an abrupt end (fig. 3). Not uncommonly, however, there was a progressive simplification of pattern—the number of spike compo-
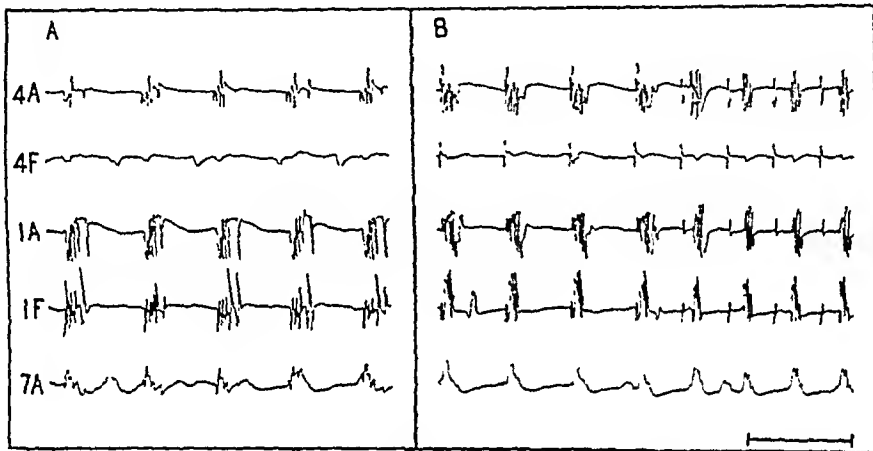
Fig. 4. Influence of rate of controlling single shocks on the clonic responses. Records: 4A, 4F, 1A, 1F, and 7A.

A. Illustrates clonic bursts in a selfsustained (undriven) response to rapid stimulation of 1A.

B. A rapid stimulus similar to that in A was applied. In addition single shocks were delivered to 6A, as shown by the stimulus artifacts (see tracing of 4F). The record illustrates the result of a sudden increase of the frequency of the single shocks: the latency of the clonic responses increases and the number of spikes in each burst decreases.

nents of the clonic complex decreased, while the slower smooth-wave component could remain unaffected or even increase in amplitude (fig. 2).

The pattern of the clonic bursts could also be affected by the rate of the controlling shocks. A characteristic example is illustrated in figure 4B. An acceleration of the driving stimuli resulted in a sudden decrease of the number of spikes in the bursts, and in addition caused an increase of the latency of the responses.

B. *Specific connections between different cortical areas.* In the previous section were considered the general characteristics of the clonic bursts evoked by single-shock stimulation of appropriate cortical areas. In this section the emphasis is on what is meant by appropriate in that context.

When a localized (non-spreading) tonic-clonic response was initiated in a given cortical region it was found that stimulation of certain other areas could control

and prolong the clonic bursts, whereas single-shock stimulation of still other areas would fail to influence the clonus.

The complete knowledge of controlling relationships in the cortex would require a study of all the regions controlled by any given area and of all the regions which can control that area. Such study was not attempted. Enough observations were made, however, to warrant the statement of the following principles.

a. There are pairs of areas in which mutual, two-way control is found. Thus, stimulation of area *4* (Brodmann, 1905) can drive clonus at area *7*, and *vice versa.*

b. There are pairs of areas which exhibit only one-way control. Thus, stimulation of area *17* can drive clonus at area *8* (fig. 5A), but stimulation of *8* does not drive the clonic responses at *17*.
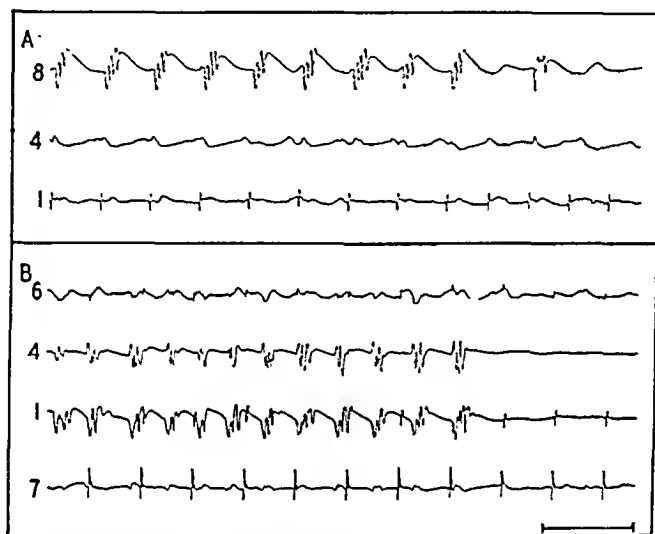
Fig. 5. Specificity of the controlling pathways.
  A. Records: *8*, *4* and *1*. Single shocks to *17* (see artifacts in *1*) control a clonus localized in the distant area *8*.
  B. Records: *6*, *4*, *1* and *7*. Single shocks to *19* (see artifacts in *7*) fail to control clonus at *4* and *1*, though *19*, like *17*, can control *8*.

c. There are pairs of areas with no mutual controlling relationships. Thus, stimulation of either *4* or *7* does not control a clonus limited to either *19* or *17*, and *vice versa* (see fig. 5B).

d. It is possible to set up 2nd or higher order control, thereby linking areas which are not directly coupled. Thus, as stated in *c*, *17* does not control *4*. Area *8*, on the other hand, controls *4*, and, as stated in *b*, *17* controls *8*. If tonic-clonic responses are set up both in *8* and *4*, stimulation of *17* may control clonus at *4* via *8*.

e. If single-shock stimulation of a given region controls the clonic activity of another, then the selfsustained clonic discharges in the first will also control the clonic activity of the second, provided that the rate of discharge of the driving area is faster than that of the driven. Figure 6 illustrates the control of clonic activity at *8* by the clonic bursts at *17* and *19*. In figure 7C, on the other hand,

19 fails to drive *8*, because the rate is slower at *19* than at *8*. Second order control is also possible in these conditions (see fig. 7D).

In addition to the controlling relationships already mentioned as examples, the following linkages were also observed. Areas *4, 1, 2* and *7* (cf. fig. 7B) all showed mutual control. This coupling was not limited to the "face", "arm" and "leg" bands which Dusser de Barenne and McCulloch (1938) described on the basis of the effects of local applications of strychnine. The face region of areas *4* or *1* could be readily driven by single shocks applied to the leg region (fig. 8), and *vice versa*, even if the arm region was not active during the tonic-clonic response.

Crossed couplings—i.e., from one hemisphere to areas on the opposite side— were also readily demonstrable. Thus, stimulation of *4* on one side controlled the clonic responses of *4, 1, 2* and *7* on the contralateral hemisphere.
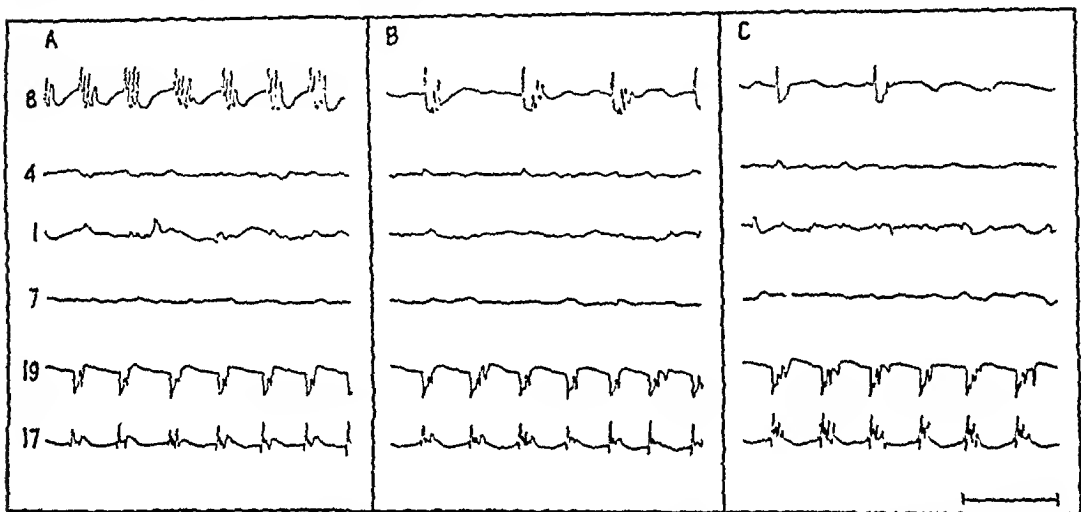
Fig. 6. Coupling of localized selfsustained clonic responses in distant areas. Records: *8, 4, 1, 7, 19* and *17*. Successive rapid stimuli were applied to areas *8* and *17*. A was taken 40 sec. after the application of the stimuli; for each clonic burst at *17* and *19* there is, with a brief delay, a corresponding clonic discharge at *8*. B, 10 sec. later; the discharges at *17* and *19* bear a 2:1 relationship with those at *8*—i.e., *8* is alternating with respect to the other areas. C, 10 sec. later; the response ends at *8* while it proceeds at *17* and *19*.

C. *The possibility of setting up two independent tonic-clonic responses in the cortex.* Since there are areas between which there is no mutual control, it was expected that separate discrete stimulation of such areas would result in responses independent of each other. This expectation was confirmed. For example, by successive stimulation of *7* and either *17* or *19*, it was possible to initiate responses with different clonic rates and durations (fig. 7A).

Even in areas with one-way coupling, independent responses could be obtained if the clonic rate of the driven area happened to be higher and the duration of the response longer than that in the driving area (cf. *19* and *8* in fig. 7C). The driving region could then not impose its rate on the driven one, much as single shocks fail to drive clonus when slower than the prevailing clonic rate (p. 682).

Occasionally, for reasons still obscure, independent responses were seen in areas usually coupled (cf. *8* with *4, 1* and *7*, in fig. 7B). A linking of independent

areas could be easily obtained by simultaneous activity in a region coupled to the others (fig. 7D). The situation was then similar to that described above when second order controls were defined.
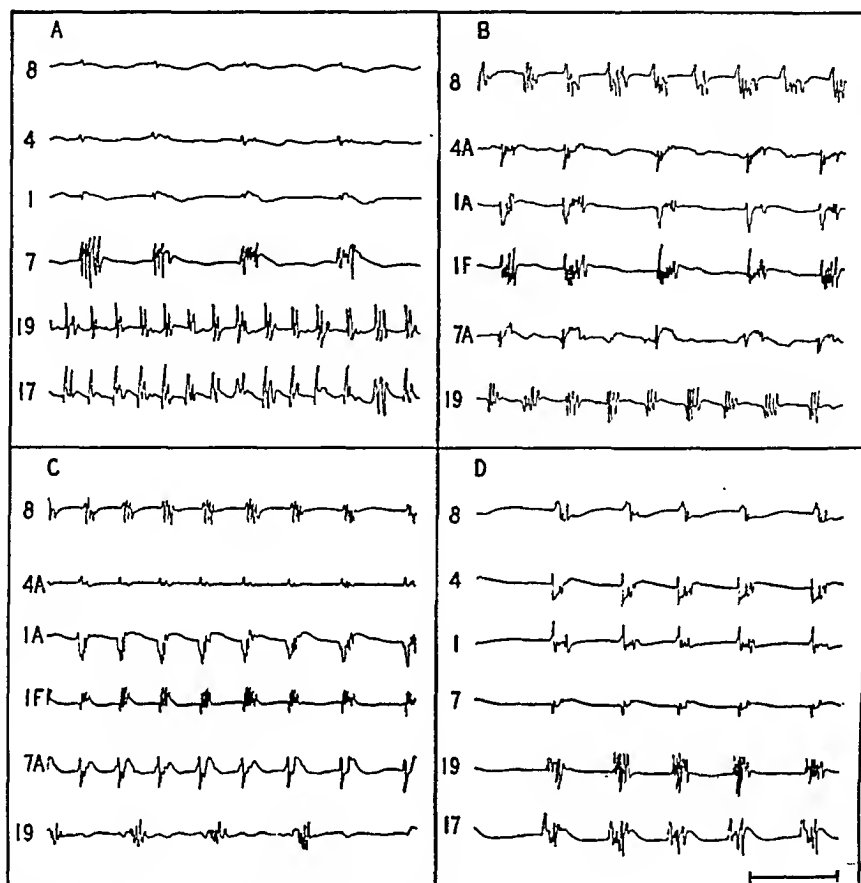
Fig. 7. Independent tonic-clonic responses in the cortex.

A. Records: *8, 4, 1, 7, 19* and *17*. Successive rapid stimulation at *17* and *7* led to two independent tonic-clonic responses, one at *7*, the other at *17* and *19*. The record shows the end of activity at *7*.

B. Records: *8, 4A, 1A, 1F, 7A,* and *19*. Rapid stimuli were applied first to *4A* and 15 sec. later to *8* and *19* in quick succession. The record begins about 20 sec. after the last stimulation. The clonus at *4, 1* and *7* is coördinated and is independent of that at *8* and *19*.

C. Records as in B. Rapid stimulation was first applied to *19*. About 30 sec. later *4* and *9* were also stimulated. The record shows the end of the response at *19*. This response was independent of the coördinated discharges in the other areas.

D. Records as in A. Successive rapid stimulation of appropriate duration at *4, 8* and *17* led to a clonic response which was coördinated throughout the cortex. The discharges at *17* lead; their control over the activity at *4, 1* and *7* is made via *8* (second order control).

D. *The effects of sections of the gray matter.* The purpose of these observations was to see whether the controlling pathways are intracortical or subcortical.

In some instances a cut was made in areas *4* or *1*, about 5 mm. deep, from the lower margin of the face region up to the midline. Single shocks were then ap-

plied to *6* arm and a clonic response was stimulated in *7* arm.   The single shocks invariably controlled the clonus at *7*, much as they had before the cut.
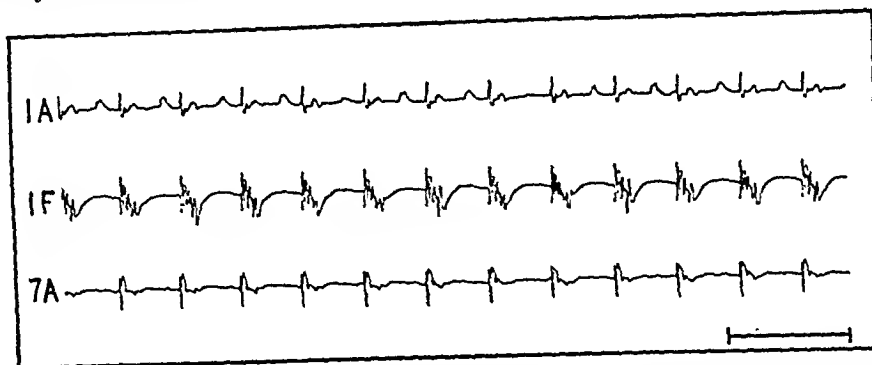
Fig. 8. Coupling of different divisions of a single area. Records: *1A, 1F* and *7A*. Rapid stimulation was applied to *1F*; it resulted in a tonic-clonic response which did not spread to the neighboring division *1A* or to other areas. The record shows the control of the clonic bursts at *1F* by single shocks applied to *1L*. Stimulus artifacts in *1A* and *7A*.
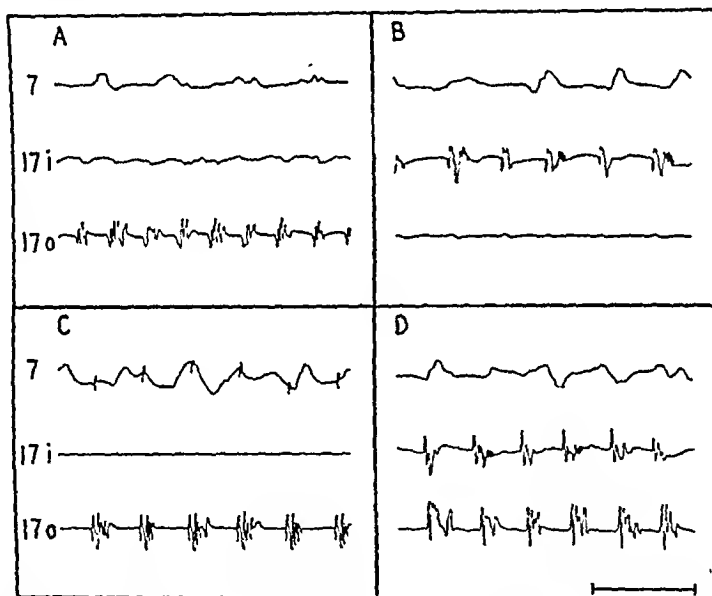
Fig. 9. Responses of an isolated cortical region in area *17*. As explained in the text, the gray matter was cut throughout the perimeter of a square with sides of about 2 cm. Records: *7, 17* inside, and *17* outside the isolated region.

A. Rapid stimulation outside caused a tonic-clonic response which did not spread inside. The clonus is illustrated.

B. As in A, but stimuli applied inside.

C. Rapid stimuli as in A. Single shocks were then applied inside and, as shown by the record, succeeded in controlling the clonic response. Each clonic burst is preceded by a diphasic stimulus artifact.

D. Two successive rapid stimulations were applied, one inside, one outside. The record illustrates the synchronism of the selfsustained clonic bursts.

In other more conclusive cases a square of gray matter with sides of about 2 cm. was entirely isolated in area *17* by *3* sections about 5 mm. deep, that included

the pia, and by an additional subpial cut, where the vessels entered the area. Records were then taken from 2 pairs of electrodes, one outside, the other inside the isolated region.   As shown in figure 9A and B, the response to rapid stimulation outside the cuts did not spread to the isolated portion, and similarly, stimulation inside did not spread beyond the sections.   Single-shocks applied inside, however, readily controlled the clonic responses of the region outside (fig. 9C), and *vice versa*.   Furthermore, if successive rapid stimulation was applied inside and outside, the clonic bursts were correlated at the two pairs of electrodes (fig. 9D).

E. *The control of clonic responses by afferent nerve impulses.*   The fact that clonic responses may be driven by electrical stimulation of the cortex naturally
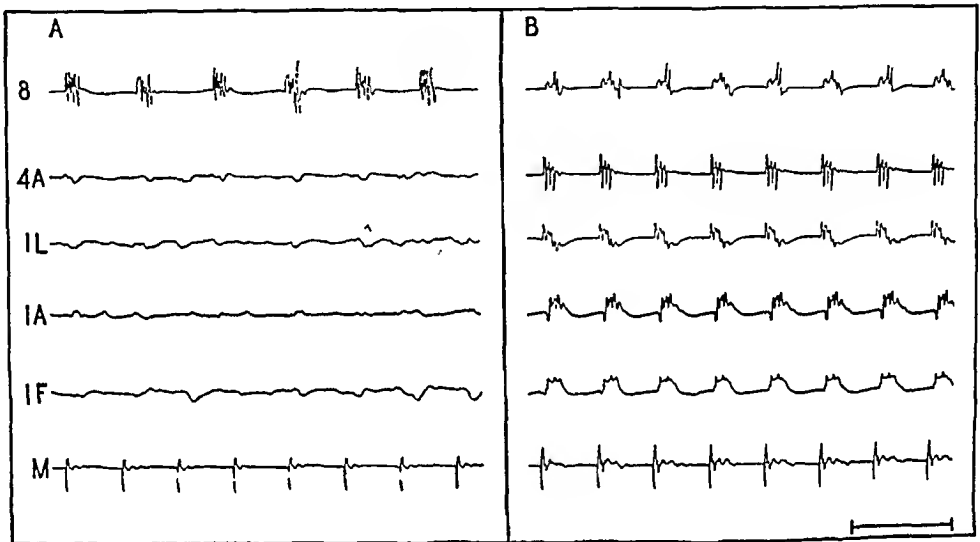
Fig. 10. Second order control in driving by afferent nerve impulses.   Records: *8, 4A, 1L, 1A* and *1F.*   The lower tracing (M) records the action potentials of a muscle activated reflexly; it indicates the time of single-shock afferent stimulation of the right sciatic.

In A sciatic stimulation fails to drive a localized clonic response at *8*.

In B sciatic stimulation drives clonus at *8* because this area is now coupled to other active regions over which the afferent impulses have a control.

suggested the possibility that centripetal impulses, set up by stimulation of afferent nerves, might also control clonic activity.

Accordingly, in some animals, in addition to the usual cortical leads, stimulating electrodes were applied to the central end of the cut right sciatic nerve.   It was possible for the afferent nerve impulses to gain control of the clonus at several areas, both in the contralateral (fig. 10B) and in the ipsilateral hemispheres. Second order control is illustrated in figure 10.   Sciatic stimulation did not influence the clonic activity at the contralateral area *8* when this activity was localized (A).   Adequate control ensued, however, when *8* was coupled to other controlled areas (B).

DISCUSSION.   I. *The similarity of the clonic responses to single shocks with the spontaneous clonic bursts.*   Throughout the description of the experimental results the assumption was made that the responses obtained by appropriate single-

shock stimulation are entirely similar to the clonic discharges of a tonic-clonic response. This assumption is based on the following considerations.

a. The responses to the single shocks, like the clonic bursts, include several spikes and a more prolonged wave, organized in a characteristic pattern (cf. fig. 4A and B).

b. The single shocks, when applied before or some time (several seconds) after a tonic-clonic response, elicit either no recordable activity or only unsustained simple responses of the type previously studied by Adrian (1936) and by Rosenblueth and Cannon (1942). Such simple responses, when present, in no aspect resemble a clonic burst. Indeed, the single shocks elicit clonic-like effects only when delivered during the clonus of the tonic-clonic sequence, not before.

c. Single shocks delivered shortly after a spontaneous clonic burst fail to elicit any typical response (p. 682). This observation leads to the inference that the same cortical elements are involved in selfsustained clonus and in the responses to single stimuli.

d. Areas which couple upon single-shock stimulation also exhibit linked undriven clonic bursts when stimulated separately (fig. 6). Conversely, uncoupled areas show independent selfsustained clonic activity (fig. 7A).

e. The rates at which electric stimulation of one region may drive another coupled region are similar to those at which selfsustained clonus occurs (p. 683).

II. *The pathways for control.* The conduction of the nerve impulses from a driving stimulated area to a driven clonically active region could *a priori* follow two different pathways. The stimulated cells could activate neighboring elements, and these in turn more outlying neurons, so that a spreading wave of activity would ensue, largely confined to the gray matter. Such waves would follow—to use a word suggested by W. S. McCulloch (personal communication) —the "feltwork" of the cortex. This mode of propagation is exhibited by some of the cortical responses to single-shock stimulation studied by Adrian (1936) and by Rosenblueth and Cannon (1942).

An alternative pathway could be long fiber tracts, with few or no relays, traveling subcortically from the driving to the driven areas. This means of conduction would ensure specificity of connections, as opposed to the indiscriminate spread which would tend to result from feltwork propagation.

That the coupling of areas is probably through long, subcortical connections is indicated by the following observations. *a.* The control is specific (fig. 5). *b.* The coupling of areas in the two hemispheres (p. 687) is undoubtedly through long pathways. *c.* Quite distant areas in one hemisphere (e.g., *17* and *8*) are coupled and one may drive the other, although it may not drive some of the intermediate regions of the cortex (fig. 5). *d.* Propagation velocity in the feltwork is relatively slow (Adrian, 1936; Rosenblueth and Cannon, 1942); yet the latency of the clonic bursts elicited in *8* by stimulation of *17* was often as brief as 25 msec. *e.* Section of the gray matter between the driving and the driven regions did not abolish the linkages between different cortical regions (fig. 9; p. 688).

In a recent study, Le Gros Clark (1941) failed to obtain any anatomical evidence of long unilateral cortico-cortical connections of area *17*. The present data give no clue as to the anatomical structure of the coupling tracts. They

indicate, however, that there are specific, long, functional pathways linking certain areas with several others.

In this and a previous study (Rosenblueth and Cannon, 1942) the spread of a tonic-clonic response in one hemisphere has always been to contiguous areas, never discontinuous—i.e., a spread to a distant area has never been observed unless the intervening regions, between the stimulated and the distant areas, first became involved in the selfsustained activity. Thus, in the present observations, although areas *17* or *19* readily controlled the clonic activity of area *8*, stimulation of *17* or *19*, no matter how strong, frequent and prolonged, never caused the appearance of a tonic-clonic response in *8*.

Tonic-clonic responses of certain cortical areas in one hemisphere can be evoked by the stimulation of appropriate regions in the opposite side (Rosenblueth and Cannon, 1942). It may be inferred, therefore, that the activation of tonic-clonic responses may be brought about via long fiber connections. To reconcile this positive datum with the negative facts in the previous paragraph it is suggested that the long nerve paths involved in the spread of a tonic-clonic response from one to the other hemisphere are different from those through which control of clonus can be obtained. Within one hemisphere predominantly or exclusively controlling pathways are present, whereas the connections between the two hemispheres involve these controlling pathways and in addition others which lead to the spread of selfsustained activity. The spread of tonic-clonic activity within one hemisphere would then be predominantly or exclusively via the feltwork. This last suggestion is supported by the failure of spread across a cut of the gray in *17* (fig. 9).

III. *Some features of cortical clonic activity.* The data reveal some interesting properties of the cortical elements involved in clonic discharges. The latency of the clonic bursts of one area in response to stimulation of another one could vary within wide ranges. For example, in a single animal the latency of the responses at *8* to stimuli applied at *17* varied from 25 to 100 msec. The changes of latency could depend on the rate of the driving stimuli (fig. 4). They could occur progressively in the course of a series of responses (fig. 2B). Finally, the latency could vary with the intensity of the driving shocks—e.g., in one observation the latency at the left area *4* of the responses to single shocks at the right symmetrical region changed abruptly from 40 to 75 msec. when the intensity of the stimuli was reduced from 27 to 17 v.

Since the conduction velocity of axons is quite constant, it is not likely that the changes in latency are due to changes of conduction velocity in the pathway which connects the stimulated with the responding regions. It appears more probable that a lengthening of latency is due to a lengthening of the synaptic delays interposed in that pathway, particularly the synaptic delay at the driven area. This interpretation covers all the changes of latency observed if the further assumption is made that the synaptic delay in question varies inversely as the excitability of the responsive cells. The progressive lengthening of latency seen toward the end of a series of clonic responses (fig. 2) would then be due to a progressive decrease of the excitability of the activated elements. The longer latency corresponding to briefer intervals between the stimuli (fig. 4) would be

due to the relative refractoriness (p. 682) of the cells (i.e., lessened excitability), when the stimuli are delivered at short intervals. The increase of latency which results from a decrease of the intensity of the driving stimuli would not be due to a change of excitability of the responding cells but to a decrease of the "density of excitation." Weak shocks stimulate fewer controlling elements than do strong shocks. The total number of nerve impulses impinging at the active area is therefore small with a weak shock—i.e., the density of excitation is low.

Rosenblueth and Cannon (1942) described two components in the electrical records of cortical clonic bursts. One of them (component III) consists of sharp, spike-like excursions, commonly multiple in the bursts. The other one (component IV) is a large round wave, usually obscured by the superimposed spikes. The prolongation of a clonic response by a series of stimuli reveals that these two components correspond to activity of different and independent elements, since often the spikes disappeared in the course of the series while component IV was still present for some time, until a sudden or gradual disappearance (fig. 2).

Changes of amplitude and duration in the clonic bursts, correlated with the frequency of clonus, occur not only when this frequency is controlled (fig. 4) but are also seen in selfsustained (undriven) clonic responses. In the course of the selfsustained discharges the frequency slows progressively and the complexity of the bursts increases correspondingly (Rosenblueth and Cannon, 1942). In nerve and cardiac or striated muscle, impulses elicited in rapid succession are subnormal in amplitude, for recovery from previous activity is not complete. There is not a strict parallelism between these simple responses and the clonic bursts. A clonic discharge involves probably single activity of some elements (component IV), but it also includes repetitive trains in others (component III). Even for this complicated pattern, however, the responses appear simplified, if sufficient time for total recovery is not allowed.

IV. *The concept of a background excitation.* To clarify this concept we shall consider specific experimental instances. Single shocks applied to area *17*, without any other stimulation of the cortex, do not give rise to clonic bursts in the ipsilateral area *8*. The same shocks, however, when applied immediately after the end of a tonic-clonic response stimulated at *8*, regularly elicit further typical clonic bursts in this region. These clonic responses may then be evoked for a long time, if the stimuli to *17* are applied above a frequency of about 1 per sec. They then will stop suddenly even though the stimuli be continued. There is no evidence of any cortical activity at *8* between the bursts produced by stimulation of *17*; indeed, even the spontaneous activity, otherwise ever present in the cortex, may be absent at the time (see Rosenblueth and Cannon, 1942). It is suggested, *a*, that the stimulation at *8*, which initiates the tonic-clonic response, builds up a prolonged state of enduring excitation in some cells of that area; *b*, that this excitation is responsible for the clonic bursts during the selfsustained response; *c*, that the excitation gradually subsides; *d*, that the progressively decreasing frequency of the spontaneous clonic bursts indicates the gradual wane of excitation; *e*, that the selfsustained discharges cease when the excitation drops below a critical level; *f*, that there still remains, however, enough background excitation so that the impulses arising at *17* succeed in tripping the clonic elements at *8*; and

g, that the end of the driven clonic bursts again indicates further wane of excitation below a second critical level.

Since a faster frequency of stimulation at *17* prolongs clonic discharges at *8* more than does a slower frequency (p. 683), it is further suggested, *h*, that each clonic burst in turn increases the background excitation in the discharging and in other similar elements. In support of this suggestion is the fact that a long tonic-clonic selfsustained response at *17* prolongs a brief tonic-clonic response at *8*—i.e., that clonic discharges at *17* may drive clonic discharges at *8* (fig. 6).

V. *The end of selfsustained tonic-clonic responses.* Two questions arise with regard to the end of a tonic-clonic response that is not prolonged by single-shock stimulation: why does the response cease?; and why does it usually cease simultaneously (Rosenblueth and Cannon, 1942) at all the active areas?

The present observations, interpreted on the basis of the suggestions outlined in the previous section, furnish an answer to these questions. When a response does not spread beyond the stimulated region the discharges cease as soon as the background excitation reaches a sufficiently low level. When a response has spread, and several areas are involved, then although the excitation may have waned at some of the regions below the level necessary for automatic discharge, such regions will remain active because they are controlled by those where excitation is still high. As long as one of the areas, which controls directly or indirectly the others, has enough background excitation for clonic activity, the response will proceed throughout, but it will suddenly stop everywhere when excitation drops below the critical level in the last controlling area.

SUMMARY

In Rhesus monkeys, under chloralose anesthesia, tonic-clonic cortical responses were elicited by rapid electric stimulation. Single shocks applied to appropriate cortical areas can control the rate of the clonic discharges and can prolong the responses beyond their intrinsic duration.

The following features of the driven clonic responses are described: alternation (fig. 1); termination of a series (figs. 2 and 3); influence of frequency (fig. 4); pattern (figs. 2 and 4); specificity of the controlling connections (figs. 5 and 8). The controlling pathways are subcortical (fig. 9). Localized tonic-clonic responses may be coupled in distant areas (fig. 6); or else they may be independent (fig. 7).

Afferent nerve impulses can control clonic discharges (fig. 10).

The discussion deals with the similarity of the driven to the undriven clonic bursts (p. 690), the controlling pathways (p. 691), some properties of clonically active elements (p. 692), the background cortical excitation (p. 693), and the factors which determine the rate and end of a clonic selfsustained response (p. 694).

REFERENCES

ADRIAN, E. D. J. Physiol. **88**: 127, 1936.
BRODMANN, K. J. f. Psychol. u. Neurol. **4**: 177, 1905.
DUSSER DE BARENNE, J. G. AND W. S. McCULLOCH. J. Neurophysiol. **1**: 69, 1938.
LEGROS CLARK, W. E. J. Anat. **75**: 225, 1941.
ROSENBLUETH, A. AND W. B. CANNON. This Journal **135**: 690, 1942.

THE EFFECT OF PERIPHERAL VASODILATATION ON VASOCONSTRICTION: DETERMINATIONS MADE ON THE BASIS OF BLOOD PRESSURE OF NORMAL SUBJECTS[1]

GRACE M. ROTH AND CHARLES SHEARD

From the Section on Clinical Physiology, Mayo Clinic, and the Division of Biophysical Research, Mayo Foundation, Rochester, Minnesota

In normal subjects elevation of the blood pressure or a vasoconstrictor response to painful stimuli has been observed by many investigators. Hines and Brown (1) have demonstrated that after the basal blood pressure has been obtained, immersion of one hand to a point just above the wrist in water at 4°C. for one minute will produce a rise of 10 to 20 mm. of mercury in the systolic blood pressure and 8 to 15 mm. in the diastolic. General anesthesia inhibited this response, derivatives of barbituric acid markedly decreased the reaction, while bromides caused only a slight decrease in it. Except for the effect of these drugs, this vasoconstrictor response over various periods was remarkably constant.

Since, for clinical purposes, 95 per cent ethyl alcohol administered orally has been used to produce peripheral vasodilatation, the question arose as to whether such peripheral vasodilatation could inhibit or alter this vasoconstrictor response of the blood pressure in normal subjects. Cook and Brown (2) have demonstrated that following the oral administration of 0.5 cc. of 95 per cent ethyl alcohol for each kilogram of body weight, the degree of vasodilatation of the vessels of the skin of the extremities of normal subjects approached that obtained with anesthesia or fever. The average maximal level reached by the surface temperature of the skin of the toes was 33.1°C. It has also been shown that when a normal subject remained for an hour or more under a hot environmental temperature of 32°C. (89.6°F.) the temperatures of the fingers and toes closely approximated the temperatures of the forehead, thorax, legs and arms, thereby indicating more or less generalized vasodilatation of the peripheral blood vessels. In order to investigate whether 95 per cent ethyl alcohol, administered orally, produced adequate peripheral vasodilatation, exposure of the normal subject to an environmental temperature of 32°C. (89.6°F.) for approximately an hour with the accompanying rise of the skin temperature of the toes could be used as comparative evidence.

The present study was made to determine whether marked peripheral vasodilatation, produced either by the oral administration of 95 per cent ethyl alcohol or by exposure of the subject to an atmospheric temperature of 32°C. (89.6°F.) for an adequate period, could alter or inhibit the vasoconstrictor response of the blood pressure of normal subjects to a painful stimulus. Since more or less generalized vasodilatation of the peripheral circulation is considered to be present

when the temperature of the skin of the toes approximates that of the skin of the fingers, and since the skin temperature of the toes is the most sensitive or delicate indicator of the vasomotor regulation of the dissipation of heat, various criteria must be maintained regarding posture, constancy of the environmental temperature and the basal state of the subject. Furthermore, higher skin temperatures of the toes are found in persons who have higher basal metabolic rates.

PROCEDURE. Data were obtained in psychrometric rooms on twelve normal subjects whose ages ranged from eighteen to forty years. The studies with alcohol took place in a room where the temperature was maintained at 25.5°C. with a relative humidity of 40 per cent. The subjects were fasted for fifteen hours previous to the tests and during the tests they wore lightweight short pajamas and were in the supine position on comfortable beds. The basal metabolic rates were first determined. Basal blood pressures were then observed and one hand was immersed in water at 4°C. for one minute and the rise of blood pressure was noted during the immersion. After the skin of the hand had returned to normal temperature, the temperatures of the plantar surfaces of the first and third toes of both feet and of the volar side of the distal phalanges of the first and third fingers of the two hands were measured by means of copper-constantan thermocouples. When fairly constant readings of the skin temperatures of the extremities were obtained, 30 cc. of 95 per cent ethyl alcohol diluted in 150 cc. of fruit juice, which was equivalent to 0.5 cc. per kilogram of body weight, was then administered orally and the blood pressure and skin temperatures were observed at ten minute intervals for one to two hours. When the skin temperature of the toes approximated that of the fingers, the same hand previously used was again immersed in water at 4°C. for one minute to determine the vasoconstrictor response of the blood pressure in the presence of dilatation of the peripheral blood vessels.

At some later time in eight of the subjects, the vasoconstrictor response of the blood pressure was again determined and the subject was placed for one to two hours in a hot room where the environmental temperature was maintained at 32°C. (89.6°F.) with a relative humidity of 40 per cent. The skin temperature of the fingers and toes was again determined and observations of blood pressure were made at intervals of ten minutes. Again, when the skin temperature of the toes approximated that of the fingers, the vasoconstrictor response of the blood pressure was repeated.

RESULTS. The basal metabolic rates of these twelve normal subjects ranged from +13 to −19 per cent. Under an environmental temperature of 25.5°C. with a relative humidity of 40 per cent, as previously mentioned, the highest skin temperature of the toes was observed among subjects who had the highest basal metabolic rates.

The average skin temperature of the toes for the group during the control period was 26.9°C. with a range from 22.5 to 31.3°C. while the average skin temperature for the fingers during the same period was 31.2°C. with a range from 27.1 to 34.5°C. The average difference between the fingers and toes was 4.3°C.

Approximately one hour after the oral administration of 30 cc. of 95 per cent

ethyl alcohol, the average maximal skin temperature of the toes was 31.8°C. with a range from 23.6 to 35.3°C. while the average maximal skin temperature of the fingers was 34.2°C. with a range from 30.3 to 36.0°C. In spite of the seeming disparity between skin temperatures of the fingers and toes, only in three instances was there a difference of 2°C. or more.

After the subjects had remained in the hot room for one hour generalized peripheral vasodilatation was evident when the skin temperatures of the toes reached the average maximal temperature of 34.2°C. with a range from 32.6 to 35.5°C. while the fingers reached an average maximum of 35.9° with a range from
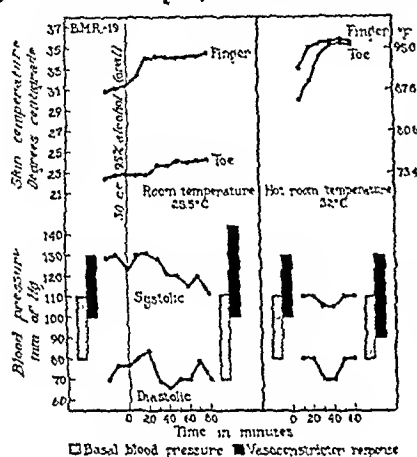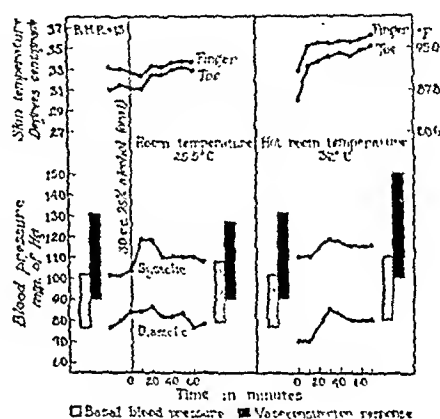
Fig. 1   Fig. 2

Fig. 1. With a basal metabolic rate of −19 per cent, the skin temperature of the toes was about 3°C. below the room temperature and one and a half hours after the administration of ethyl alcohol it did not approximate that of the fingers. A difference of more than 10°C. existed between the skin temperatures of the fingers and toes. After the subject had been exposed to the high environmental temperature, the temperature of the skin of the toe approximated that of the finger. The vasoconstrictor response of the blood pressure was slightly higher after the administration of ethyl alcohol in this subject than with the high environmental temperature.

Fig. 2. With a basal metabolic rate of +13 per cent the skin temperature of the toes and the fingers was considerably higher than room temperature and the temperature of the toes readily approximated that of the fingers with both the administration of ethyl alcohol and subjection to a high environmental temperature. In this subject, the vasoconstrictor response of the blood pressure was slightly higher with the hot environmental temperature than after administration of ethyl alcohol.

35.4 to 36.1°C. The average maximal temperatures were slightly higher under the increased environmental temperatures than with 95 per cent ethyl alcohol.

During the control period, the average basal blood pressure in millimeters of mercury for the group was 104.3/73, with a range of 90/50 to 120/94. During the immersion of one hand up to the wrist in water at 4°C. for one minute the blood pressure rose to an average in millimeters of mercury of 124/88 with a range of 102/74 to 140/100. The average increase was 19 mm. of mercury in the systolic and 15.4 mm. in the diastolic.

Approximately one hour after the oral administration of 30 cc. of 95 per cent ethyl alcohol when the temperature of the skin of the toes demonstrated more or

less generalized peripheral vasodilatation, the average basal blood pressure in millimeters of mercury for the group was 101.6/71 with a range from 90/55 to 122/90. Immediately following this observation, the hand was immersed in water at 4°C. for one minute. During immersion the average blood pressure rose to 121/87 with a range from 94/70 to 144/100. The average increase was 20.7 mm. of mercury in the systolic and 16.5 mm. in the diastolic. This response of the blood pressure was similar to that produced during the control period. At another time the average basal blood pressure in millimeters of mercury of eight of the subjects, during a control period, was 102.2/69 with a range from 90/55 to 110/75. After exposure of the subjects to an environmental temperature of 32°C. (89.6°F.) for between one and two hours and when the temperature of the skin of the toes demonstrated generalized peripheral vasodilatation the average basal blood pressure for the group was 98.8/66.3 mm. of mercury, with a range of 90/55 to 110/80. The hand was immersed again in water at 4°C. for one minute, and during immersion the blood pressure in millimeters of mercury rose to an average of 123.9/87.0 with a range of 108/75 to 150/100. The slight changes which took place could not be considered significant.

A composite picture of some of the variations of the skin temperatures and blood pressures is given for two subjects (figs. 1 and 2), one with a basal metabolic ıate of −19 per cent and the other with a basal metabolic rate of +13 per cent.

SUMMARY

In these twelve normal subjects, irrespective of the basal metabolic rate and irrespective of the existing generalized peripheral vasodilatation, the response to the vasoconstricting agent was not altered significantly.

REFERENCES

(1) HINES, E. A. AND G. E. BROWN. Ann. Int. Med. 7: 209, 1933.
(2) COOK, E. N. AND G. E. BROWN. Proc. Staff Meet., Mayo Clin. 6: 449, 1932.

THE PRODUCTION OF EXPERIMENTAL POLYCYTHEMIA BY THE DAILY ADMINISTRATION OF EPINEPHRINE OR POSTERIOR PITUITARY SOLUTION[1]

JOHN EMERSON DAVIS

From the Department of Physiology and Pharmacology, University of Arkansas School of Medicine, Little Rock, and the University of Vermont, College of Medicine

We have previously reported the production of experimental polycythemia in dogs, rabbits and man by the daily administration of appropriate doses of ephedrine sulfate. (1) Amphetamine sulfate was also found to be effective in augmenting erythropoiesis in dogs (1) and in man (2). The results were attributed to local hypoxia of bone marrow due to the curtailment of its blood supply through the vasoconstrictor action of these drugs.

The work to be reported here was performed in an effort to determine whether other well known vasoconstrictor drugs could produce polycythemia, when given daily in appropriate doses. Epinephrine and solution of posterior pituitary were the drugs selected for this experiment. It is known that polycythemia often accompanies human cases of pituitary basophilic adenoma, and it is possible that this polycythemia might be due to an associated hyperactivity of the adrenal medulla. High doses of pituitrin have been shown to cause experimental "pituitrin anemia" in rabbits (Dodds et al., 3, and Gilman and Goodman, 4), which is due to hemolysis of red cells caused by dilution of the serum electrolyte as a result of water retention.

PROCEDURE. Dogs and rabbits were used in this investigation. They were maintained on constant adequate diets and were allowed water ad libitum. Control observations were made on the blood of each animal during a period of 3 weeks prior to drug administration. Red cell counts were made regularly together with estimations of hemoglobin percentage (Hellige). Total leukocyte counts were also made at intervals.

Solution of posterior pituitary was injected subcutaneously into one splenectomized and two normal dogs in daily doses of 5 and 10 units. The same preparation was given to 4 normal and 2 splenectomized rabbits in daily subcutaneous doses of 0.5, 1.0 and 2 units, for about 15 days. Epinephrine hydrochloride was injected subcutaneously into one splenectomized and two normal dogs in daily doses of 0.5, 1.5 and 2 mgm. Epinephrine was also injected daily into 3 normal and 2 splenectomized rabbits in doses ranging from 0.1 to 0.3 mgm.

During the experiment, blood samples were drawn only after an interval of at least 18 hours following the last drug injection. Precautions were taken to keep the animals as free from excitement as possible. Blood was drawn by syringe from the external saphenous veins of the dogs; in the rabbits, blood was drawn directly into diluting pipettes from the site of puncture of a marginal ear vein.

[1] Research paper no. 531, journal series, University of Arkansas.

After about 18 days the drug administrations were stopped, and in most instances observations on the blood were continued for about 2 weeks thereafter.

RESULTS. The daily subcutaneous injection of 0.5 to 2 units of solution of posterior pituitary into 4 normal and 2 splenectomized rabbits resulted in significant increases (11 to 20 per cent) in their basal erythrocyte numbers within 9 to 14 days (fig. 1). The animals receiving the lowest dose of posterior pituitary (0.5 unit) showed the least increase of red cells. The counts returned to normal in 10 to 15 days following the cessation of drug injections.

The red cell counts of one splenectomized and two normal dogs were increased by 15 to 21 per cent by the daily subcutaneous injection of 5 to 10 units of solution of posterior pituitary for 10 to 17 days (fig. 2). Hemoglobin percentages increased correspondingly, but the total leukocyte counts did not change significantly. The erythrocyte counts in two of the dogs returned to normal in about 10 days following cessation of drug administrations. Unfortunately, the
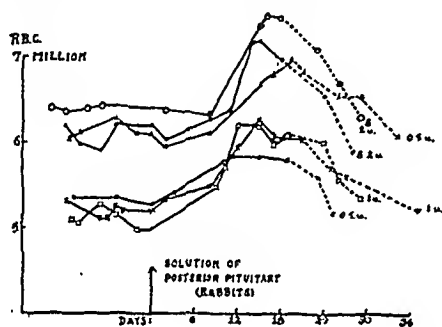
Fig. 1   Fig. 2
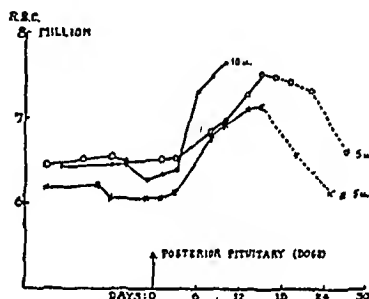
Fig. 1. The effect of posterior pituitary solution on four normal and two splenectomized rabbits. S indicates splenectomized rabbit. Values at the end of each curve give the daily subcutaneous dose in units for each animal. Dash lines indicate cessation of drug administration.

Fig. 2. The development of polycythemia by dogs receiving posterior pituitary solution subcutaneously. Values at end of each line indicate the daily dose of drug for that particular animal (u = units). Dashes indicate discontinuation of drug injections. S indicates a splenectomized dog.

third dog (fig. 2), while very healthy, was accidentally allowed to escape from the animal house, and we were therefore unable to study the return of his erythrocyte count to normal.

Epinephrine hydrochloride was injected subcutaneously into 3 normal and 2 splenectomized rabbits in daily doses ranging from 0.1 to 0.3 mgm. As may be seen in figure 3, this procedure caused gradual increases in their erythrocyte numbers which became maximal after 12 to 18 days of drug injection. After cessation of epinephrine administration, the red cell counts returned to normal within about 15 days.

Figure 4 shows the development of polycythemia in one splenectomized and 2 normal dogs which received daily subcutaneous injections of epinephrine hydrochloride. The doses given were 0.5, 1.0 and 1.5 mgm., daily. After 15 days of drug injections, these animals showed increases in their red blood cell counts of 12 to 21 per cent. The dog which received the highest daily dose of

epinephrine showed the least increase of red cells. Hemoglobin percentages changed correspondingly with the erythrocyte count, but total leukocyte counts showed no uniform or constant change. Red cell counts in all 3 dogs returned to normal within 10 to 15 days after cessation of epinephrine administration.

DISCUSSION. The results indicate that epinephrine and posterior pituitary probably caused an increased erythropoiesis in these experiments. Our strongest reason for this belief is to be found in the slow development of polycythemia (figs. 1–4) and the slow recovery from the same after discontinuation of the drugs. The time relationships correspond generally with those involved in the production of polycythemia by exposure to low atmospheric pressure (5).

The delay in production and recovery from polycythemia also argues against the possibility that our results might be due to blood concentration or to contraction of blood reservoirs. The relative constancy of the total leukocyte counts observed in our experiments on dogs constitutes additional evidence
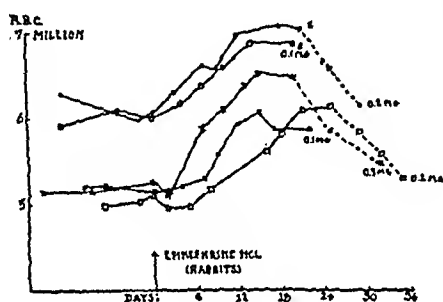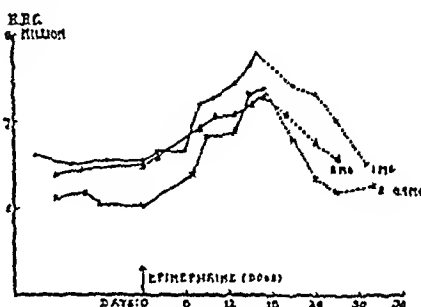
Fig. 3   Fig. 4

Fig. 3. The production of polycythemia in rabbits by the daily subcutaneous injection of epinephrine hydrochloride. S indicates splenectomized animals, and values at the end of each line show the daily dose of drug for each rabbit. Dashes signify discontinuation of drug injections.

Fig. 4. The effect of daily subcutaneous injections of epinephrine on the erythrocyte numbers of dogs. S indicates a splenectomized dog. Values at end of each line indicate the daily drug dose. Dashes signify discontinuation of drug administration.

against the possibility of concentration of the blood. The fact that polycythemia is induced as readily in splenectomized as in normal animals (figs. 1–4) also suggests that blood reservoirs probably are not concerned in the development of polycythemia caused by epinephrine or posterior pituitary.

The most likely mechanism by which epinephrine and posterior pituitary increase hemopoiesis is probably through the creation of a local hypoxia of bone marrow. This would probably be brought about through diminution of the blood supply to the marrow by the vasoconstrictor action of the drugs.

It is perhaps permissible to speculate that the results of these experiments may explain the mechanism of the polycythemia which is sometimes observed in pituitary basophilism or Cushing's disease in humans.

CONCLUSIONS

The daily subcutaneous administration 0.5 to 2.0 units of posterior pituitary solution to four normal and two splenectomized rabbits caused significant

increases in their erythrocyte numbers within 9 to 14 days. One splenectomized and two normal dogs also showed significant polycythemias after the subcutaneous administration of 5 to 10 units of posterior pituitary solution, daily, for 10 to 17 days.

The daily subcutaneous injection of 0.1 to 0.3 mgm. of epinephrine hydrochloride into 3 normal and 2 splenectomized rabbits resulted in gradual increases in their erythrocyte counts which became maximal after 12 to 18 days. One splenectomized and two normal dogs also developed significant polycythemias within about 15 days following the onset of daily injections of 0.5 to 1.5 mgm. of epinephrine hydrochloride.

These results are explained by assuming that epinephrine and posterior pituitary cause increased erythropoiesis by inducing a local hypoxia of bone marrow. This is presumably accomplished through reduction of the blood supply to the marrow as a result of the vasoconstrictor action of these drugs.

REFERENCES

(1) Davis.   This Journal **134**: 219, 1941.
(2) Davis.   This Journal **137**: 94, 1942.
(3) Dodds and Noble.   Nature **135**: 788, 1935.
(4) Gilman and Goodman.   This Journal **118**: 241, 1937.
(5) Davis.   This Journal **127**: 322, 1939.

THE EFFECT OF ANOXIA ON BRAIN POTENTIALS
OF HYPERTHYROID ANIMALS

M. KESSLER and E. GELLHORN[1]

From the Departments of Physiology and Psychiatry, College of Medicine, Chicago, Illinois

The effect of thyroid administration on the oxygen uptake of excised brain is well established (Cohen and Gerard, 1937; Rossiter, 1940). Several authors (Streuli, 1918; Barach, Eckman and Molomut, 1941) have shown that thyroid administration increases and thyroidectomy decreases the sensitivity of various laboratory animals to anoxia. Therefore, it seemed worthy to investigate the effect of thyroid hormones on the electroencephalographic changes induced by anoxia.

METHODS. The experiments were performed on unanesthetized rats of approximately 250 grams weight. One group of the animals was injected with 0.1 to 0.2 mgm. thyroxine/100 gram weight for 9 days. Another group received 0.2 mgm. thyroxine/100 gram weight for 4 days. The third group was given thyroid powder U. S. P. (Armour and Co.) 100 mgm. per day for 12 days. Whereas the thyroxinized rats lost some weight it was not appreciably altered in the rats which were treated with thyroid powder. Anoxia was produced either by allowing the rats to inhale seven per cent oxygen from Douglas bags or by exposure to lowered barometric pressure of 280 and 255 mm. Hg. The E. E. G. was recorded using phonograph needle electrodes (Hoagland) through an Offner crystograph.

RESULTS. It has been pointed out in an earlier study (Gellhorn and Kessler, 1942) that no significant changes in E. E. G. are observed when normal rats are exposed to 7 per cent oxygen. However, thyroxinized rats (fig. 1) show profound changes in the E. E. G. under similar conditions. In the first experiment of figure 1 alpha potentials disappear more or less completely and slow delta waves appear. This effect is reversible on readmission of air. In the second experiment in which the control shows some delta waves the administration of 7 per cent O_2 greatly accentuates the delta potentials. Then the alpha potentials decrease in size and finally the brain waves disappear almost completely. In this case also the phenomenon is reversible.

The experiments involving the administration of thyroid powder were less effective than those performed with thyroxine. However, definite quantitative differences appeared between control and hyperthyroid animals. In 8 control experiments only two animals showed a definite transient increase in delta potentials at the lowered barometric pressure whereas the remaining 6 animals did not show any significant changes in the E. E. G. In the experimental group all animals showed on exposure to lowered pressure electroencephalographic changes which consisted either of a transient or a progressive increase in

delta potentials or of a complete disappearance of brain waves. This shows that electroencephalographic changes were more frequent and more severe in the experimental than in the control group.
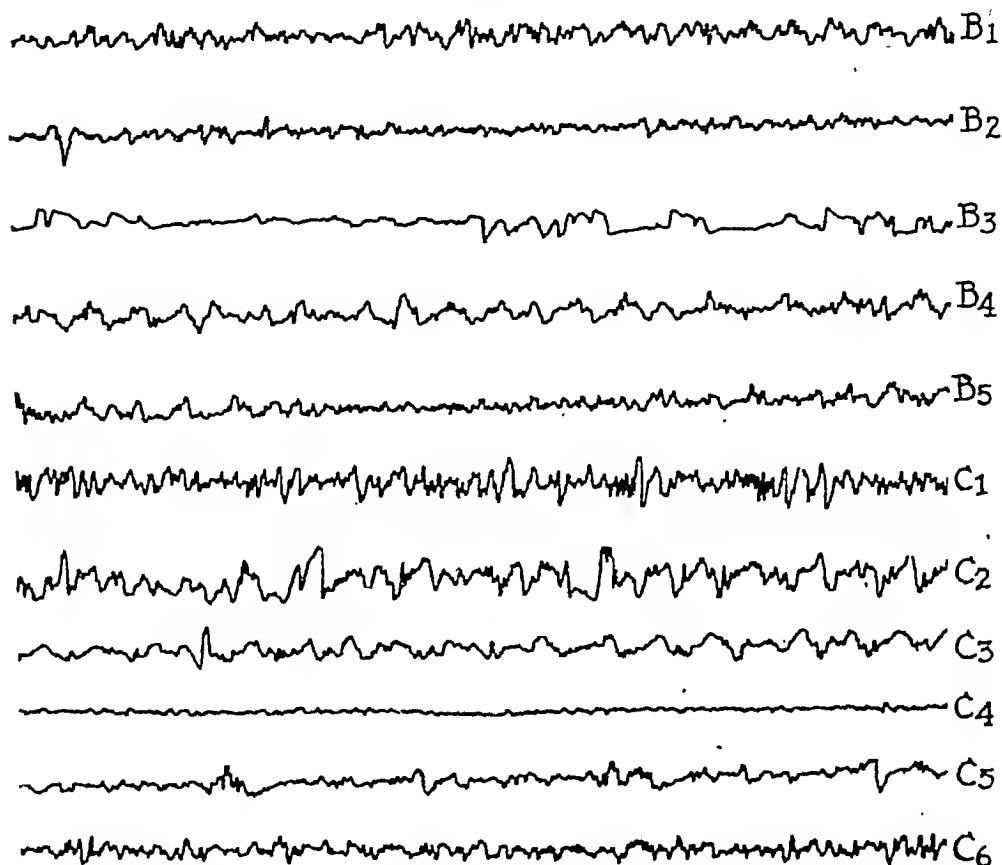
Fig. 1. The effect of 7 per cent O_2 on the E. E. G. of unanesthetized rats injected with thyroxinc. B_1 control; B_2 and B_3 after one and three minutes of 7 per cent O_2 respectively. B_4 and B_5 1 and 4 minutes after readmission of air.

C_1 control in air; C_2 and C_3 after $2\frac{1}{2}$ and 3 minutes of 7 per cent O_2.
C_4, C_5 and C_6, 2, 3 and 9 minutes after readmission of air.
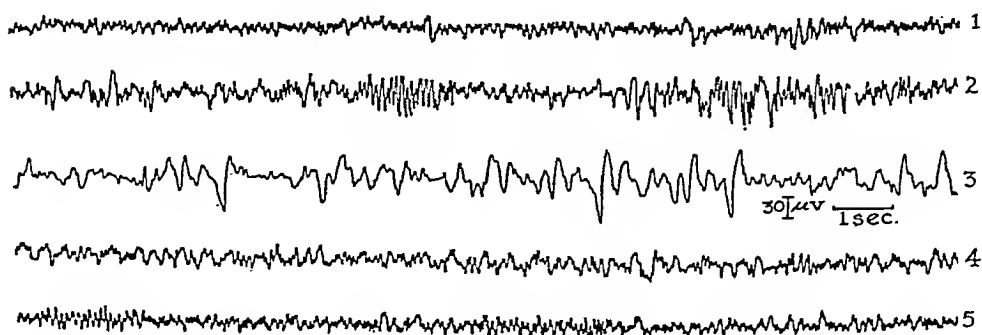
Fig. 2. The effect of lowered barometric pressure (280 mm. Hg) on the E. E. G. of rats which were given thyroid powder by stomach tube.

1, control at normal barometric pressure; 2, 3, 4: 1, 7 and 12 minutes at 280 mm. Hg; 5, 10 minutes after readmission of air.

Figure 2 shows a marked increase in delta potentials accompanied by a decrease in alpha potentials at 280 mm. Hg. It is interesting to note that these effects were only transient (cf. records 3 and 4 of fig. 2) whereas the changes induced by anoxia (fig. 1) in thyroxinized rats were always progressive.

Apparently the sensitivity to anoxia as demonstrated by electroencephalographic changes is related to the degree of hyperthyroidism since anoxia produced the greatest changes in rats which had suffered loss of weight from the administration of thyroid hormones.

It should be added that the E. E. G. of the hyperthyroid animals at normal oxygen tension was unchanged.

SUMMARY

The administration of thyroid powder or of thyroxine increases the sensitivity of unanesthetized rats to 7 per cent O_2 or lowered barometric pressure as shown by the greatly accentuated effects of anoxia on the electroencephalogram.

REFERENCES

BARACH, A. L., M. ECKMAN AND N. MOLOMUT. Am. J. Med. Sci. 202: 336, 1941.
COHEN, R. A. AND R. W. GERARD. J. Cell. Comp. Physiol. 10: 223, 1937.
GELLHORN, E. AND M. KESSLER. This Journal 136: 1, 1942.
ROSSITER, R. J. J. Endocrinol. 2: 165, 1940.
STREULI, H. Biochem. Ztschr. 87: 359, 1918.

THE INFLUENCE OF ANTACIDS UPON IRON RETENTION BY THE ANEMIC RAT

SMITH FREEMAN AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

An efficient utilization of dietary factors concerned in hemoglobin formation is of particular importance to the individual with a chronic ulcer of the stomach or duodenum. Such lesions may result in an excessive blood loss and some of the diets used are low in substances contributing to hemoglobin formation. Antacids are frequently administered in relatively large amounts to this group of patients, and it is possible that certain antacids may affect the absorption of iron from the intestine.

Kletzein (1) has shown that calcium carbonate as well as various other carbonates reduce iron retention in the anemic rat. He suggested (2) that the Ca-P ratio in the diet might be an important factor in influencing iron absorption. Anderson, McDonough and Elvehjem (3) studied the effect of various Ca-P ratios in the diet upon iron absorption by anemic rats and found that a low ratio favored iron absorption while a high Ca-P ratio resulted in a low assimilation of iron. A previous report (4) from this laboratory suggested the likelihood of aluminum hydroxide contributing to the anemia observed in dogs subjected to the Mann-Williamson operation. Data were presented at the same time which showed that aluminum hydroxide interferes with the absorption of phosphorus in dogs as well as in human subjects maintained on a "light ulcer diet."

The present study was undertaken to determine the effect of some "antacids" commonly employed in the treatment of chronic gastric and duodenal ulcer upon iron retention by the anemic rat. The relative dosage of the various anti-acids studied was chosen arbitrarily on the basis of what seems to be commonly employed comparable therapeutic doses.

EXPERIMENTAL PROCEDURE. *Animals.* Wistar strain rats were made anemic according to the method of Elvehjem and Kemmerer (5). When the animals were definitely anemic (hemoglobin 2–3 grams), each litter was divided into two groups with equal distribution of sexes and so that the average hemoglobin content of each group was similar. All animals were continued on a milk diet but the main daily feeding was withheld until all supplements were completely consumed. Both the control and the ant-acid-fed group received daily 0.25 mgm. iron as ferric chloride, 0.05 mgm. of copper and of manganese as their sulphates. These supplements were fed in a small quantity of milk. Aluminum hydroxide and aluminum phosphate were fed as a 5 per cent and 4 per cent suspension, respectively; the suspension was mixed with an amount of milk that the animal was certain to consume and the control group was likewise restricted. Calcium carbonate and magnesium trisilicate were fed as a dry powder mixed with finely

powdered cane sugar. The control group received an equal amount of cane sugar. All antacids used were shown to contain insignificant quantities of iron. The relative amounts of the various antacids fed were determined from the following considerations: a fairly liberal antacid regimen for an ulcer patient might require 200 cc. of 5 per cent aluminum hydroxide daily, 250 cc. of 4 per cent aluminum phosphate, or 6 grams of either calcium carbonate or magnesium trisilicate. The average iron requirement for man is considered to be in the vicinity of 10 mgm. daily. Then a comparable intake of iron and antacid would be in these proportions. Twenty-five hundredths milligram of iron daily has been shown (6) to cause marked increase in hemoglobin in the anemic rat. This amount does not represent an excess of iron and the organism is likely to utilize it as efficiently as possible. This amount of iron is approximately one-fortieth the daily dosage employed for man, e.g., 5 cc. of aluminum hydroxide, 6 cc. of aluminum phosphate, and 0.15 mgm. of calcium carbonate or magnesium trisilicate.

Each animal was caged separately. The cage consisted of an inverted bottomless 3-gallon bottle with a platform made of no. 2 mesh galvanized wire that was heavily tinned after construction of the platform. The platform had a diameter of 7.75 inches. Each cage had a wooden lid with numerous small perforations covered with gauze so that the cage is essentially fly-proof. This cage has the advantages ascribed to both glass and metal cages, namely, little chance for iron contamination or for refection to occur.

METHODS. Hemoglobin was converted to alkaline hematin, according to the method of Wu (7) and read in a Klett-Summerson photoelectric colorimeter using filter no. 54. The white cell pipette used for measuring the blood for all determinations of hemoglobin was standardized on a sample of dog's blood whose hemoglobin content was calculated from its iron content (8). Inorganic phosphorus was estimated by Bodansky's modification (9) of the Kutner-Lichtenstein procedure (10).

The extent of depletion was indicated by the hemoglobin content of the blood. After depletion the animals were maintained on the supplements for 26 to 28 days and then killed by ether inhalation. The entire carcass was ashed in a muffle furnace, at 1000°F., dissolved in hydrochloric acid, and evaporated to dryness twice, redissolved in hydrochloric acid, and then made up to a definite volume with iron-free water and analyzed for iron (8). The color was estimated in a Klett-Summerson photoelectric colorimeter using filter no. 54 and a series of standard solutions. One group of animals was killed and analyzed at the end of the depletion period.

EXPERIMENTAL RESULTS. The results show (table 1) that the depleted rats contain approximately 1 mgm. of iron per rat. After 26 to 28 days on a daily supplement of 0.25 mgm. of iron, the entire carcass of the control group averaged from 4.26 to 4.56 mgm. per rat. There is close agreement between the various control groups. The antacids that most definitely reduced iron retention were aluminum hydroxide and calcium carbonate. The difference between the iron content of the control and experimental groups for either of these antacids is

quite consistent and striking. The critical ratio of the difference in iron content of the aluminum hydroxide-fed group and its control group is statistically significant. The same is true of the calcium carbonate-fed group. The hemoglobin content of the blood at the end of the experiment is in satisfactory agreement with the iron content of the carcass for the various groups and conveys the same impression as one derives from results on iron content. The iron content of the magnesium trisilicate-fed group is somewhat lower than that of the control group and the critical ratio indicates that the difference may be significant. The hemoglobin increase in this group was also less than for the control group. Aluminum phosphate causes no decrease in iron retention or in hemoglobin formation.

TABLE 1

SUBSTANCE	NUMBER OF ANIMALS		WEIGHT OF ANIMALS		WEIGHT INCREASE			TOTAL MGM. OF IRON PER RAT			AVE. INIT. Hb IN GMS./100 CC.	Hb INCREASE IN GMS./100 CC.			IRON RETEN. PER CENT OF IRON FED
	Male	Female	Ave. init. wt.	Ave. final wt.	Mean in gms.	Stand. dev.	Crit. ratio	Mean in mgms.	Stand. dev.	Crit. ratio		Mean in gms.	Stand. dev.	Crit. ratio	
Aluminum hydroxide.....	7	3	86.5	131	44.5	4.44		2.97	0.156		2.60	2.92	0.323		30.6
Control....................	6	4	83	128	44.6	4.51	0.014	4.26	0.187	7.678*	2.72	5.71	0.260	6.755*	49.0
Aluminum phosphate.....	4	6	84	146	61.2	4.41		4.52	0.190		2.69	5.18	0.421		52.3
Control.......	3	7	75	124	48.6	3.10	2.337*	4.56	0.176	0.154	2.93	4.76	0.723	0.502	53.6
Calcium carbonate	3	8	63	133	69.5	5.13		3.43	0.090		2.49	4.79	0.293		37.0
Control.	3	9	62	126	64	4.83	0.780	4.38	0.151	5.398*	2.54	6.46	0.272	4.195*	51.0
Magnesium trisilicate......	4	7	61	117	56	4.99		3.90	0.231		2.84	5.61	0.292		43.8
Control..	4	7	62	130	67	4.195	1.687	4.50	0.152	2.172*	2.86	6.16	0.216	1.519	52.8
Depleted group.....	6	4	75					0.94			2.60				

* Indicates significance as determined from J. P. Guilford's *Psychometric methods* (1st ed., McGraw-Hill, 1936). Significant critical ratios are indicated in table K, p. 548. Statistical method is described on pp. 44–51 inclusive.

DISCUSSION. The results obtained in the present experiments are in agreement with the observations mentioned in the introduction. Fifteen-hundredths of a gram of calcium carbonate daily is enough calcium to alter the ratio of calcium to phosphorus in the diet of rats consuming from 30 to 60 cc. of milk daily from 1.2 to approximately 2 or 3 depending upon the amount of milk consumed. Aluminum hydroxide ingestion will increase the calcium-phosphorus ratio by reducing the available phosphorus. The extent to which this reaction will occur depends upon a number of variable factors (4). That the effect upon phosphorus absorption was not sufficient to deplete definitely the organism is indicated by the fact that the phosphorus content of four aluminum hydroxide-fed rats varied from 0.650 to 0.680 gram per 100 grams of carcass, averaging 0.663 gram, while four litter mate controls ranged from 0.670 to 0.727 gram per

100 grams of carcass with an average value of 0.694. However, it is the ratio of calcium-to-phosphorus in the intestine which seems to be of importance rather than the absolute amount of phosphorus that is absorbed. Since milk is a relatively phosphorus-rich food, there might be considerable impairment in its absorption without producing a depletion of the body's stores of this element. The relatively slight effect of magnesium trisilicate upon iron retention may be because there is relatively less magnesium present or because this element does not interfere with phosphorus absorption to a sufficient degree. The lack of effect of aluminum phosphate on iron retention might be due either to the fact that it cannot react further with phosphorus or because it tends to stabilize the reaction of the stomach contents at a lower pH (4). It is not pertinent to comment concerning the relative effect of these substances upon the reaction and secretion of the stomach and intestines.

From the data presented it is apparent that ingested iron is less efficiently utilized when the intake of calcium carbonate or aluminum hydroxide is high. To a lesser extent the same tendency is manifested by magnesium trisilicate. The impairment in iron retention could probably be overcome by the ingestion of additional iron. It appears reasonable to conclude that the iron intake of individuals ingesting relatively large quantities of these substances should be greater than for the average individual. This would be particularly important if accompanied by an excessive blood loss.

SUMMARY

It has been shown that calcium carbonate and aluminum hydroxide definitely reduce iron retention by the anemic rat ingesting 0.25 mgm. of iron daily. Magnesium trisilicate reduced iron retention somewhat but to a degree that is of questionable significance. Aluminum phosphate did not reduce iron retention. It is suggested that the iron intake needs to be greater in individuals consuming increased amounts of aluminum hydroxide or of calcium carbonate, than in the normal subject.

REFERENCES

(1) Kletzein, S. W. J. Nutrition Supp. 9: 9, 1935.
(2) Kletzein, S. W. J. Nutrition Supp. 15: 16, 1938.
(3) Anderson, H. D., K. B. McDonough and C. A. Elvehjem. J. Lab. and Clin. Med. 25: 464, 1939–1940.
(4) Fauley, G. B., S. Freeman, A. C. Ivy, A. J. Atkinson and H. S. Wigodsky. Arch. Int. Med. 67: 563, 1941.
(5) Elvehjem, C. A. and A. R. Kemmerer. J. Biol. Chem. 93: 189, 1931.
(6) Smith, M. C. and L. Otis. J. Nutrition 13: 573, 1937.
(7) Wu, H. J. Biochem. (Japan) 2: 173, 1922.
(8) Elvehjem, C. A. J. Biol. Chem. 86: 463, 1930.
(9) Bodansky, A. J. Biol. Chem. 99: 197, 1932–33.
(10) Kutner, T. and L. Lichtenstein. J. Biol. Chem. 86: 671, 1930.

THE PRODUCTION OF SHOCK BY TRAUMA AFTER SPINAL CORD TRANSECTION[1]

A. E. SCHECTER,[2] M. L. CULLEN[3] AND N. E. FREEMAN

With the technical assistance of M. DRESBACH, C. KRAUL and M. K. LAWS

From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia

In previous experiments it was shown that a decrease in plasma volume resulting in shock could be produced experimentally by reducing the peripheral circulation. The decrease in blood flow resulted either from hemorrhage (1) or through injecting massive doses of adrenalin (2). It was further shown that when the extremities of dogs were traumatized under ether anesthesia a reduction in peripheral circulation preceded other signs of oncoming shock (3). Compression of the extremities by bandage and adhesive tape restricted the local fluid loss but did not prevent the reduction in blood volume as shock came on after trauma. Following recovery from total sympathectomy, trauma no longer caused a reduction in circulation but still a loss of blood volume was observed, greater than could be accounted for by the accumulation of fluid in the injured area (3). It was felt that the use of ether as an anesthetic might have influenced the results and accordingly the trauma experiments were repeated in dogs after recovery from spinal cord transection. Under these circumstances it was not necessary to use a general anesthetic.

METHODS. The spinal cord was transected between the first and second thoracic vertebrae in eight mongrel dogs. The animals were studied two or more days after recovery from this operation. The carotid artery and trachea were cannulated under local anesthesia. Oxygen consumption and arterial and venous oxygen and carbon dioxide concentrations were measured. The cardiac output was calculated by the Fick principle and blood volume determinations by the carbon monoxide method were made before and within three hours after trauma. Blood pressure was recorded from the carotid artery by means of a mercury manometer. Peripheral blood flow through an uninjured paw was measured by the plethysmograph. Hemoglobin and hematocrit determinations were made on blood obtained from an ear. The muscles of one hind leg were traumatized by 1,000 blows with a rubber hammer, according to the technic of Best and Solandt (4), and the bones of the extremity were fractured with a heavy metal bar. Blood loss into the area of injury was restricted by binding the extremity as suggested by Freedman and Kabat (5). The loss of blood into the area of injury was measured by the dissection technic of Cullen and Freeman (6) at the termination of the experiment. The visceral organs were examined after death.

RESULTS. Four control experiments were performed. One of these was discarded because of the poor condition of the animal due to wound infection and diarrhea. In the other three, as shown in table 1, there were no significant changes in the volume or character of the circulation during the experimental period. The course of a single control experiment is shown in figure 1.

In comparison with these results, after bone and muscle trauma a loss of blood volume was consistently observed. This loss of blood volume occurred within three hours after trauma while the circulation was still well maintained. Figure 2 shows the results obtained in the most striking experiment. The blood pressure and peripheral blood flow were not seriously reduced immediately after the trauma in three of the four experiments. A moderate reduction in cardiac

TABLE 1

DATE	EXPER. NO.	DOG NO.	WT.	DAYS AFTER TRANSECTION	BLOOD VOLUME			LOCAL FLUID LOSS	HEMOGLOBIN		HEMATOCRIT		BLOOD PRESSURE		BLOOD FLOW		CARDIAC OUTPUT		VENOUS OXYGEN SATURATION	
					Before trauma	After trauma	Difference		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
			kgm.		*cc.*	*cc.*	*cc.*	*gm.*	%	%	%	%							%	%
Control experiments																				
6-23	1	1026	14.2	6	1508	1346	162		82	84	30.5	34.5	140	105	2.2	2.4	8000*	3008	52.3	68.3
7-17	2	13	8.6	2	1166	991	175		91	91	18.6	18.5	94	96	6.0	4.0	1763	2035	66.0	80.0
7-10	3	1		2	1120	1118	2		93	95	35.2	35.2	110	96	10.7	11.3	1500	1319	59.6	59.6
7-14	4†	3	9.9	5	1931	928	1003		72	79	24.4	34.2	110	104	3.8	4.7	3015		62.7	
Trauma experiments																				
	5	2	11.8		2125	1029	1096	40	65	98	25.9	42.5	128	54	4.2	1.2	2520	737	60.7	45.2
7-24	6	7	9.0	16	1362	909	453	115	74	80	27.0	34.0	92	104	3.1	1.7	2170	882	68.9	41.0
6-27	7	1038	10.6	3	984	623	361	65	90	98	29.0	34.0	124	102	9.0	7.0	1226	868	50.0	47.2
6-25	8	1037		5	1600	1015	585	85	12.0 (O₂ capacity)	12.9	21.0	24.5	80	94	1.3	2.7	1993	1501	47.8	33.5

* Dog panting. Arterial oxygen saturation reduced.
† Experiment excluded because of the presence of wound infection and fever.

output was observed at the end of the experimental period in two of the dogs while it was marked in the other two.

DISCUSSION. It is agreed that tissue anoxia from reduced circulation causes capillary damage with consequent loss of plasma volume. The present experiments were designed to study the effect of trauma on blood volume where the injurious effects of reduced circulation were avoided. In addition, the possible complicating influence of a general anesthetic was eliminated by using dogs after spinal cord transection.

Reduced circulation was excluded in the present experiments by transecting the spinal cord between the first and second thoracic segments. By this procedure, not only were pain stimuli from the area of injury prevented, but also reflex efferent vasoconstrictor impulses were eliminated. These reflexes might have originated from unavoidable loss of blood or from fear and might have

caused splanchnic vasoconstriction. Reduced circulation from excessive local fluid loss also was avoided by binding the injured areas.

It is true that adequacy of the circulation to the viscera and to the muscles cannot be inferred from observations on the blood flow through the paw. The maintenance of cardiac output and oxygen saturation of mixed venous blood in three of the experiments, however, suggests that the general circulation was adequate during the experimental period. The moderate decrease in cardiac output in experiments three and four might well be attributed to the loss of blood volume. Repeated measurements of the peripheral circulation during the period after trauma, and before the second measurements of cardiac output and blood volume were made, suggested that the reduction in blood volume preceded the decrease in circulation.
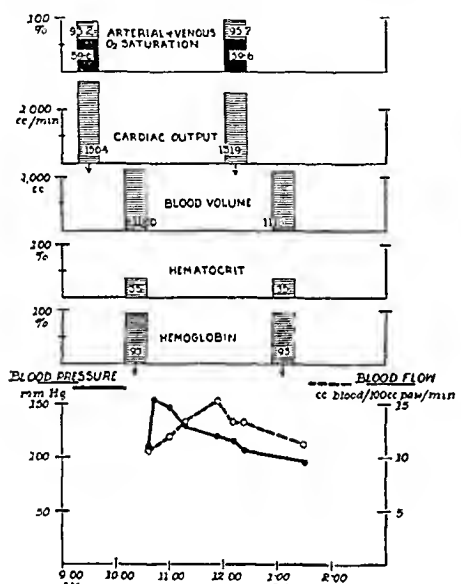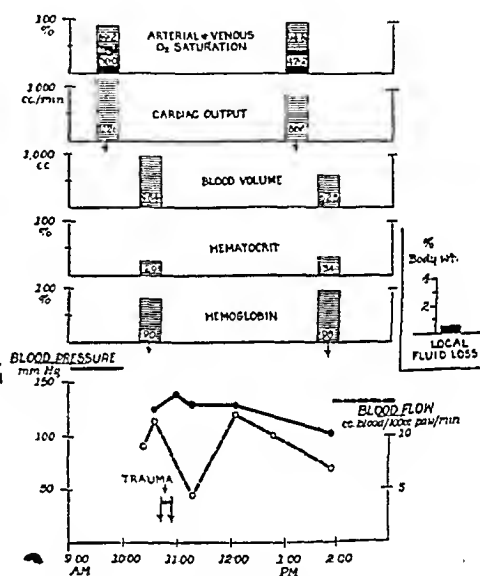
Fig. 1   Fig. 2

Fig. 1. Effect of experimental procedures on the circulation of the dog after spinal cord transection, without trauma.

Fig. 2. Effect of trauma on the circulation of the dog after spinal cord transection.

In experiment 1 the blood pressure and blood flow were seriously depressed shortly after the trauma. It is possible that the reduction of blood volume in this experiment might have resulted from the circulatory defect. The possibility of fat embolism was considered as the cause of this fall in blood pressure but no fat droplets could be found in the pulmonary capillaries on microscopic examination of the lungs. That fat embolism could not have been etiologically significant in producing shock in the other experiments was shown by the fact that the blood pressure did not fall immediately after the trauma.

The effect of exclusion of sensory impulses upon the development of shock has been studied by various workers with different results. Simonart (7), O'Shaughnessy and Slome (8) and more recently Freeman and Kabat (5), believed that nervous impulses were of great significance, while Parsons and

Phemister (9), Freedlander and Lenhart (10), Holt and MacDonald (11), and Blalock (12) believed that nervous impulses were of secondary importance in the genesis of shock. It seems possible that the differences in opinion depend in part upon variations in the amount of blood and fluid unavoidably lost at the site of injury and upon the use of different criteria of shock. In experiments previously reported on the effects of trauma in sympathectomized dogs we judged, according to criteria of blood pressure, blood flow and even of recovery, that the process of shock had not been initiated. When we measured the blood volume, however, we found that it was reduced more than could be accounted for by the loss into the traumatized area. Shock is essentially the process of loss of blood volume due to increased capillary permeability. Only by adopting the criteria of reduced blood volume or increased capillary permeability is it possible to say whether or not the process has been initiated.

Objection may be raised that the most striking experiment with trauma was selected for comparison with a control experiment. This selection was made since in this case, above all, a significant reduction in blood volume occurred in spite of a circulation which was apparently adequately maintained. The evidence in the remaining experiments supported the concept to which we had been led. Furthermore, the present experiments confirm the observations which were made upon the reduction of blood volume after trauma in sympathectomized dogs. Under those circumstances reduced circulation was again not a significant factor.

Post mortem examination disclosed congestion of the mucosa of the upper intestinal tract with edema of the intestinal wall and even some free fluid in the lumen of the gut. These findings are characteristic of shock. Exactly by what mechanism this loss of blood volume occurred is obscure. It was present, although less pronounced, even in the two dogs which were sacrificed while their circulation was still adequate. When reduced circulation persisted for an hour or more, the course of shock appeared to be hastened and the pathological changes in the viscera were more intense.

SUMMARY AND CONCLUSIONS

1. Bone and muscle trauma was inflicted upon four dogs after recovery from spinal cord transection.

2. Four control experiments without trauma were performed.

3. The adequacy of circulation was determined by measurements of blood pressure, peripheral blood flow, cardiac output and oxygen content of mixed venous blood.

4. The amount of fluid loss into the traumatized area was measured and was kept at a low level by binding the injured extremity.

5. A reduction of blood volume and hemoconcentration occurred after trauma in the presence of a well-maintained circulation and in the absence of excessive blood loss into the injured extremities.

6. Post-mortem examinations revealed pathological changes characteristic of shock.

7. These findings suggest the action of some factor capable of causing a reduction in blood volume not primarily due to excessive local fluid loss or to reduced circulation.

REFERENCES

(1) FREEMAN, N. E., S. A. SHAFFER, A. E. SCHECTER AND H. E. HOLLING. J. Clin. Investigation 17: 359, 1938.
(2) FREEMAN, N. E., H. FREEDMAN AND C. C. MILLER. This Journal 131: 545, 1941.
(3) CULLEN, M. L., A. E. SCHECTER, N. E. FREEMAN AND M. K. LAWS. Blood substitutes and blood transfusion. C. C. Thomas, Springfield, Ill.. 1942.
(4) BEST, C. H. AND D. Y. SOLANDT. Proc. of the Am. Physiol. Soc., 1941. p. 25.
(5) FREEDMAN, A. M. AND H. KABAT. This Journal 130: 620, 1940.
(6) CULLEN, M. L. AND N. E. FREEMAN. Surgery 10: 770, 1941.
(7) SIMONART, A. Arch. Internat. de Pharmacol. 37: 269, 1930.
(8) O'SHAUGNESSY, L. AND D. SLOME. Brit. J. Surg. 22: 589, 1935.
(9) PARSONS, E. AND D. P. PHEMISTER. Surg., Gynec. and Obst. 51: 196, 1930.
(10) FREEDLANDER, S. O. AND C. H. LENHART. Arch. Surg. 25: 693, 1932.
(11) HOLT, R. L. AND A. D. MacDONALD. Brit. Med. J. 1: 1070, 1934.
(12) BLALOCK, A. Arch. Surg. 20: 959, 1930.

NITROGEN CLEARANCE FROM THE BLOOD AND SALIVA BY OXYGEN BREATHING

P. F. SCHOLANDER AND G. A. EDWARDS

From the Edward Martin Biological Laboratory, Swarthmore College, Swarthmore, Pennsylvania

Several investigators have shown that the course of the elimination of nitrogen from the body during the breathing of pure oxygen follows an exponential curve (Campbell and Hill, 1931, 1933; Behnke, Thomson and Shaw, 1935; Behnke and Willmon, 1941). The latter authors found that 40 to 50 per cent of the total nitrogen was eliminated during the course of the first hour, whereas 80 to 90 per cent clearance required 4 to 5 hours. No direct determinations of the nitrogen content of blood and saliva during clearance seem to have been reported in the literature. The present paper describes the nitrogen clearance from human blood and saliva during a period of breathing pure tank oxygen, as determined by a micro-gasometric technique. Small samples allowed frequent determinations both conveniently and accurately.

TECHNIQUE. The measurements of the nitrogen content of the blood and saliva were made by means of a method for the determination of nitrogen in small amounts of blood and saliva and other fluids (Scholander, 1942). Samples of 40 cu. mm. of saliva were obtained anaerobically from the ducts of the sublingual glands. Each subject was in a resting position during the experiment, and was provided with a nose mask through which he could breathe pure tank oxygen. Samples of blood and saliva were taken and analysed at frequent intervals before, during, and after the period of oxygen breathing.

RESULTS. The figure shows three curves of the nitrogen clearance from the finger blood and two curves of the nitrogen clearance from the saliva. Four different subjects were used. The samples of normal blood and saliva contained an average of 1.02 volume per cent nitrogen, with a range from 0.97 to 1.05 volume per cent. The curves show that the nitrogen was eliminated rapidly from both finger blood and saliva during the period of oxygen breathing. Eighty to 90 per cent of the nitrogen was cleared within the first 10 minutes. The remaining 10 to 20 per cent was gradually eliminated during the next 50 minutes, after which time the oxygen mask was removed. At the end of 1 hour the nitrogen content of the blood and saliva of the subjects had reached the low value of 0.06 volume per cent. Beyond this point the analytical procedure and sampling were less certain on account of the smallness of the bubble to be measured and the danger of air contamination of the samples. When the subjects breathed air again after the removal of the mask, the nitrogen in the blood and saliva usually showed a transitory rise above normal. This excess in recovery was also noticed in preliminary experiments, but the reason for its occurrence is undetermined. The rate of resaturation of the blood and saliva with nitrogen

from the air was practically as rapid as the rate of desaturation during the oxygen breathing, and both processes occurred within 10 minutes. The clearance figures for finger blood nitrogen and saliva nitrogen did not show any significant differences.

The experiments show that the nitrogen is cleared rapidly from both finger blood and saliva as compared with the very slow total clearance, as determined by Behnke and Willmon (1941). The clearance curves for the finger blood and for the saliva are practically identical and are probably close to the clearance
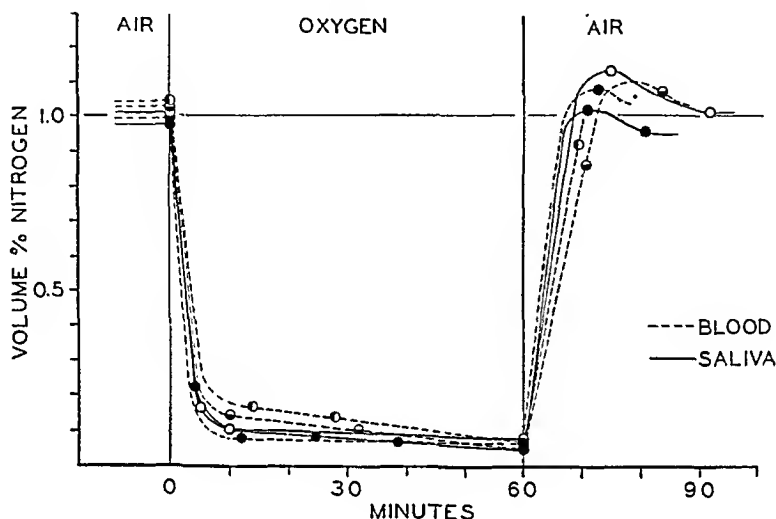
Fig. 1. Curves of the elimination of nitrogen from the finger blood and the saliva of four different persons at rest. The subjects are represented by the various types of circles.

curves of arterial blood. They are probably more closely indicative of the alveolar nitrogen than of the tissue nitrogen.

We wish to express our appreciation to Dr. Laurence Irving for his help and support.

REFERENCES

BEHNKE, A. R., R. M. THOMSON AND L. A. SHAW. This Journal 114: 137, 1935.
BEHNKE, A. R. AND T. L. WILLMON. This Journal 131: 619, 1941.
CAMPBELL, J. A. AND L. HILL. J. Physiol. 71: 309, 1931.
        Quart. J. Exper. Physiol. 23: 197, 1933.
SCHOLANDER, P. F. Review of Scientific Instruments, in press.

MECHANISM OF THE EFFECT OF EPINEPHRINE ON THE VENOUS HEMATOCRIT VALUE OF THE NORMAL UNANESTHETISED DOG

P. F. HAHN, W. F. BALE AND J. F. BONNER, JR.[1]

From the Departments of Pathology and Radiology of the University of Rochester School of Medicine and Dentistry

We submit evidence below to show that hemoconcentration as indicated by an increase in the venous hematocrit reading does not necessarily represent a change in the mass of the circulating red blood cells. By the use of red cells tagged with the radioactive isotope of iron[2] we have found that there are no large reserve stores of red cells in the dog but that essentially all of the cells are in active circulation (5). Stead and Ebert by measurement of the hemoglobin removed by phlebotomy and comparison with the changes in circulating hemoglobin as determined indirectly with plasma volume estimations arrived at the conclusion that such was also the case in human subjects (8). The data presented herein are in accord with these findings and suggest that a rearrangement of the distribution of vascular components must be considered in obtaining an adequate conception of the status of the circulatory system at a given time.

That the intravenous administration of epinephrine sometimes results in an increase in the peripheral red cell count and the venous hematocrit value was pointed out in 1915 by Lamson (7), who felt that the change was due to liberation of cells stored by the liver. Barcroft and his associates (1) (2) did not subscribe to the idea that the liver acted as an important storehouse for red cells but stated that the spleen served in this capacity. Furthermore Barcroft (2) as well as Cannon and Izquierdo (3) found that although the administration of adrenalin did not invariably increase the peripheral red count, it did cause contracture of the spleen. The net result of these observations has been the generally accepted assumption that when the drug epinephrine is given by vein the resultant apparent increase of the red cells in the circulation is due to liberation of cells due to splenic contracture.

With a means of differentiating between red cells in active circulation and those which might be temporarily sequestered by splanchnic or other storage, the above concept should readily lend itself to test. Circulating red cells may be tagged effectively by the addition of other cells to the circulation, the latter having been formed in a donor animal of the same species, the hemoglobin of which has some of its constituent iron in the form of the radioactive isotope (5) (6). If the total radioactivity of the injected cells is known it becomes pos-

sible to measure the circulating mass of red cells of the recipient after allowing sufficient time for mixing of the cells.   If the spleen holds an appreciable number of red cells from the active circulation, administration of adrenalin, by contraction of this organ, should result in a further measurable dilution of the circulating red cell radioactivity.   A clear cut demonstration would necessarily be dependent on a minimal rate of circulation of red cells in the spleen during the period of cell mass estimation.

METHODS.   Duplicate blood samples were drawn from the jugular veins of adult dogs.   Twenty-five milliliters of whole citrated or heparinised blood containing a known amount of red cell radioactivity were injected into the same vein in a period of about fifteen seconds.   After time for complete mixing (6), usually four or five minutes, 25 ml. of blood were withdrawn from the same vein into 5 ml. of isotonic (1.4 per cent) sodium oxalate.   One-half milliliter of epinephrine (1:1,000) was then injected through the same needle and after three minutes another 25 ml. of blood were taken.   In some instances another injection of donor blood was carried out with subsequent sampling after four or five minutes.   There was no stasis at any time except during the initial venepuncture.   The needle was allowed to remain in the vein throughout the experiment and was occasionally flushed with blood from the circulation by alternately withdrawing and reinjecting a few milliliters of blood with a syringe. Sampled blood was divided into three parts for triplicate determination of radioactivity content and hematocrit measurement.   Blood was centrifuged for thirty-five minutes at about 2500 r.p.m.   Ashing of the red cells, separation and electroplating of the contained iron, and determination of the radioactivity were carried out as described elsewhere (4).

Radioactive iron was prepared by the method of Wilson and Kamen (9).

EXPERIMENTAL OBSERVATIONS.   A preliminary series of injections of epinephrine in the same dosage as used in these experiments showed that under these conditions the elevation of venous hematrocrit reading was rather a transient phenomenon.   The maximum level was reached in the neighborhood of one minute and the hematocrit usually had reached its initial level in five minutes. Cell mass was not measured after one minute however since it would not allow time for complete mixing.   Therefore a compromise was made on a three minute sampling, but it must be kept in mind that this might well have affected the degree of responses shown in tables 1 and 2.

Under these conditions the increase can be seen not to have been an invariable effect obtained following epinephrine injection.   In general the response was consistent and of about the same degree in the same dog.   Some animals repeatedly showed minimal changes and obviously were of no interest for the study of relationship between cell mass and hematocrit changes.

In table 1 it is to be noted that even in the animals in which a fairly uniform increase in hematocrit follows adrenalin injection, when the animals were splenectomised the response was abolished.   In fact in no animal studied in which splenectomy had been performed have we observed such a response.   Except for the evidence in table 2, in which there is found no relationship between the

red cell mass as directly determined and the venous hematocrit changes, such a finding would be construed as further evidence that the splenic contraction following adrenalin normally liberated red cells to the active circulation causing an increased hematocrit value. We are not prepared to say on a basis of the evidence available what the correct explanation may be. It is conceivable that the spleen acts in some altogether different fashion in conjunction with epinephrine to affect the distribution of the vascular constituents. An assumption that the circulation rate through the spleen is of the same order of magnitude as that of the general circulation, and that the effect of adrenalin is to cause a preferential loss of red cells from the splenic pulp while retaining a considerable amount of plasma might explain the findings, but there is no evidence to support this here.

It should also be pointed out that the adrenalin response does not seem to be related to the degree of anemia in these dogs, although unexpectedly the greatest hematocrit value changes occurred in the extremely anemic animals, 39–196 and 39–299, table 1. From a viewpoint of splenic storage it would be difficult to conceive of an animal with a severe anemia having a reserve of red cells withheld from the active circulation.

DISCUSSION. We may consider in what the mechanism of increased hematocrit values following adrenalin may consist. Since the red cells of the dog are all in active circulation in the normal and anemic animal (5) (6) the various fractions of the vascular fluids which we must consider are then: the red cell mass (CV), the volume of plasma in rapid circulation (PV_c), and the volume of plasma in sluggish circulation (PV_s). The normal partition of these fractions has been discussed in some detail elsewhere (6). The changes in the distribution of these constituents in which we might find an increased venous hematocrit reading are then:

1. An increase in CV with no change in PV_c.
2. An increase in CV with an accompanying smaller increase in PV_c.
3. A decrease in CV with a greater decrease in PV_c.
4. No change in CV with an accompanying decrease in PV_c.

Since with the administration of adrenalin it is shown that CV does not change greatly we may assume that condition four above best fits the findings under these circumstances. This would, in view of the rather rapid resumption of the hematocrit level to its former value after adrenalin injection, indicate a temporary movement of some of the PV_c fraction to the PV_s. It seems doubtful if this decrease in rapidly circulating plasma takes place through loss of fluid from the vascular system. If it does it should be accompanied by an increased plasma protein concentration which was unfortunately not followed in these experiments. However the transient nature of the reaction argues against the quite sudden loss and gain of vascular fluid within a period of five minutes or less.

If the effective number of blood vessels of small inside diameter were markedly increased, or if by vasoconstriction the caliber of a large number of vessels were decreased, the effect would be to increase the amount of plasma in sluggish circulation (6). This would seem to be a more reasonable explanation of the

TABLE 1

Venous hematocrit changes following the injection of epinephrine by vein

DOG	RED CELL HEMATOCRIT			DOG	RED CELL HEMATOCRIT		
	Initial	After adrenalin	Rise		Initial	After adrenalin	Rise
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
40-183	26.8	36.1	29.8	40-115 splenect.	31.8	31.5	−0.9
40-183	38.3	49.2	28.4	40-115 splenect.	45.7	45.1	−1.3
40-183	48.4	55.6	14.9				
				40-194 splenect.	38.9	40.0	2.8
30-299	42.4	49.8	17.5				
30-299	36.0	40.9	13.6	36-196	13.6	19.6	44.2
30-299	11.9	16.4	37.8	36-196	23.0	22.6	−1.7
				36-196	26.3	34.0	29.2
41-455	53.4	55.5	3.9	36-196 splenect.	29.5	31.5	6.8
				36-196 splenect.	32.9	34.6	5.2
41-557	43.8	45.2	3.2				
				1-G	41.4	49.8	20.2
40-15	30.8	36.2	17.5	1-G splenect.	36.7	37.4	1.9
				1-G splenect.	38.1	38.5	1.1
4-E	45.7	47.2	3.3				
4-E	42.2	42.7	1.2	2-G	46.1	48.4	5.0
4-E	23.3	23.9	2.6	2-G splenect.	28.2	29.1	3.2
4-E	22.5	22.3	−0 9	2-G splenect.	30.4	32.2	5.9
4-E	12.0	12.5	4.2				
39-57	56.0	59.8	6.8				
39-320	23.2	29.6	27.6				
39-320	30.3	35.6	17.5				
39-320	44.5	51.9	16.6				
39-320	48.8	58.8	20.5				
39-320 splenect.	36.8	36.5	−0.8				
39-320 splenect.	38.1	38.4	0.8				

TABLE 2

Relative constancy of red cell mass with increased venous hematocrit following adrenalin

DOG	RED CELL HEMATOCRIT			RED CELL CIRCULATING MASS		
	Initial	After adrenalin	Rise	Initial	After adrenalin	Rise
	per cent	*per cent*	*per cent*	*ml.*	*ml.*	*per cent*
39-320	44.5	51.9	16.6	347	347	0
39-299	36.0	40.9	13.6	366	369	0.8
39-196	26.3	34.0	29.2	234	244	4.3
39-196	13.6	19.6	44.2	125	129	3.2
39-196 splenect.	32.9	34.6	5.2	321	340	6.2
1-G	41.4	49.8	20.2	470	490	4.3
1-G splenect.	36.7	37.4	1.9	518	542	4.6

reaction especially when the nature of the pharmacological action of epinephrine is recalled. We should not lose sight of the possible change in vascular set-up which results from partial constriction of vessels such that most of the red cells in the axial stream are washed out leaving what amounts to tubes of cell free plasma. Such blood vessels are probably contracted to somewhat less that 7 microns and would contribute greatly to the fraction of plasma in sluggish circulation.

If there is a movement of plasma from the rapidly circulating to the sluggish moving state as a result of adrenalin administration the effect is similar to that which would be obtained by the removal of plasma from the active circulation and therefore the use of adrenalin in certain types of shock might be contra-indicated.

Reasoning teleologically we might say that such a shift in the rapidly moving plasma fraction resulting in a hematocrit reading increase might constitute a useful mechanism whereby in an emergency when epinephrine is poured into the circulation by physiological response, an increase in gas exchange is facilitated. The reaction being quite transitory the distribution of the plasma and red cells is then quickly restored to a normal state.

SUMMARY

When epinephrine is administered by vein in single large doses to adult intact dogs there is sometimes a decided increase in venous hematocrit readings. Some dogs uniformly show a marked increase whereas others rather consistently show minimal changes.

In the animals in which there is an increase in venous hematocrit reading there is no corresponding change in the circulating red blood cell mass as directly determined by means of the donor-isotope-red cell procedure.

Since splenectomy abolishes the hematocrit response to epinephrine and since no reserve cells are found to be poured into the circulation following adrenalin, another mechanism than outpouring of reserve red cells from the contracted spleen must be postulated to account for the increase in venous hematocrit value following the administration of this drug.

A suggested explanation of the phenomenon may be an increase in the effective number of blood vessels of small inside diameter as well as a reduction in the caliber of many vessels by vasoconstriction, both factors leading to an increased fraction of plasma in sluggish circulation.

REFERENCES

(1) BARCROFT, J. Lancet 1: 319, 1925.
(2) BARCROFT, J. AND P. ROTHSCHILD. Arch. int de pharm. et therap. 38: 569, 1930.
(3) CANNON, W. B. AND J. J. IZQUIERDO. This Journal 84: 545, 1928.
(4) HAHN, P. F., W. F. BALE AND W. M. BALFOUR. This Journal 135: 600, 1942.
(5) HAHN, P. F., W. M. BALFOUR, J. F. ROSS AND G. H. WHIPPLE. Science 93: 87, 1941.
(6) HAHN, P. F., J. F. ROSS, W. F. BALE, W. M. BALFOUR AND G. H. WHIPPLE. J. Exper. Med. 75: 221, 1942.
(7) LAMSON, P. D. J. Pharmacol. and Exper. Therap. 7: 169, 1915.
(8) STEAD, E. A., JR. AND R. V. EBERT. This Journal 132: 411, 1941.
(9) WILSON, R. R. AND M. D. KAMEN. Physic. Rev. 54: 1031, 1938.

THE DISTRIBUTION OF SUCROSE IN BODY FLUIDS FOLLOWING INTRAVENOUS INJECTIONS

ROGER S. HUBBARD AND RICHMOND K. ANDERSON

From the Buffalo University School of Medicine and the Edward J. Meyer Memorial Hospital, Buffalo, New York

In recent years the osmotic effects of hypertonic sucrose solutions have been widely utilized in a variety of conditions, particularly for the reduction of increased intracranial pressure and for the production of diuresis.

Articles on the clinical use of sucrose repeatedly state that sucrose possesses an advantage over other sugars such as glucose because it does not readily pass through capillary walls, does not enter most body fluids and is not absorbed by body tissues. This supposed non-diffusibility of sucrose is frequently contrasted to the rapid diffusibility of glucose and saline solutions. The experimental evidence does not support these conclusions. Keith, Power and Peterson (1) found that in dogs, a large percentage of the injected sucrose disappeared rapidly from the blood stream and could be detected in the tissues. Lavietes, Bourdillon and Klinghoffer (2) found that sucrose was distributed in approximately the same fraction of the body fluids as thiocyanate and inorganic sulfate, and could be used as a measure of extracellular fluid volume. Hubbard and Terplan (3) were able to detect sucrose in the spinal fluid following intravenous injection. In a preliminary report we presented evidence that sucrose entered readily into certain other body fluids (4).

We have attempted to clarify the confusion regarding the diffusibility of sucrose by studying its distribution in patients with abnormal accumulations of extracellular fluids and by comparing their rate of eliminating sucrose from the blood with that of a control group.

This study has been considerably facilitated by the use of a differential fermentation method for sucrose which has enabled us to measure very small amounts of sucrose with considerable accuracy.

METHODS. Our control group consisted of ten patients admitted to this hospital for a variety of conditions but without evidence of renal or circulatory failure and without edema or other abnormal fluid accumulations. The second group included nine patients with anasarca, edema, ascites or pleural effusions from a variety of causes as shown in table 3.

Each patient received an intravenous injection of 60 cc. of 50 per cent sucrose in a period of approximately five minutes. Reactions were few and seldom alarming. Sterile blood samples were taken at the intervals shown in the tables. Sterile body fluids were collected by paracentesis, thoracentesis or by the use of large needles inserted subcutaneously in edematous cases.

Sucrose was determined by the resorcinol method of Roe (5) after differential fermentation using cultures of B. coli communis (Escherichia coli) and B. coli

communior (Escherichia communis). The latter will ferment glucose, sucrose and other sugars while the former will ferment glucose and other sugars but not sucrose. The difference between the two samples, therefore, represents the sucrose concentration. Sterile samples (usually 2 cc.) of serum or fluid were heavily inoculated with cultures of B. coli communis and communior respectively and incubated twenty-four to forty-eight hours. Previous to inoculation, all samples were cultured for sterility and any found contaminated were discarded. Following incubation a protein free filtrate was prepared by the zinc method of Somogyi (6) using a dilution of 1:10 for the serum and 1:5 for other body fluids. The sucrose concentration in the filtrate was then determined by the resorcinol method of Roe for fructose, using a series of sucrose standards of varying dilution. Those above 0.5 mgm. per cent were read in a colorimeter. Lower values were estimated by direct comparison.

The blank in the communior sample (from which all sugars had been removed by bacterial action) was subtracted from the color in the communis sample. This blank almost invariably gave a color less than that equivalent 0.5 mgm. per cent of sucrose.

TABLE 1

Recovery of sucrose added to serum and to ascitic fluid

BLOOD SERUM		ASCITIC FLUID	
Calculated	Determined	Calculated	Determined
84	92	36	37
42	42	11	12
17	16	2	1
2	2		

We found that the values obtained by the Roe method were similar when samples were hydrolyzed and compared with fructose standards and when compared with sucrose standards without hydrolysis. Therefore, sucrose standards were used and hydrolysis omitted throughout this study.

Identical colors were obtained with and without centrifuging after inoculation to remove suspended bacteria, showing that the presence of the organisms themselves did not affect color production. Cultures of B. coli communior were added to glucose and sucrose containing sera and identical blanks obtained, indicating that any difference in the fermentation of these sugars did not influence color development. Identical blanks were also obtained when each organism was added to glucose-containing sera indicating that any differences in the metabolism of these two strains of the organism did not influence the determination.

We were able to obtain good recovery of sucrose added to serum and to ascitic fluid as shown in table 1.

RESULTS. 1. *Study of the rate of disappearance of sucrose from the blood stream in patients without evidence of renal or circulatory failure and without abnormal*

fluid accumulations. Following the injection of sucrose, blood was taken at varying intervals in different individuals. The results in the ten patients have been combined in table 2. It will be seen that following an initial high level, there is an early rapid drop followed by a more gradual fall, only traces being present after twenty-four hours, the serum containing no sucrose after forty-eight hours. These findings are in accordance with those of Keith and Power who were able to recover eighty-nine to ninety-eight per cent of injected sucrose within twelve to twenty-four hours after injection (7).

2. *Study of the distribution of sucrose in patients with abnormal extracellular fluid accumulations.* The results in patients with abnormal fluid accumulations are in marked contrast to the controls. An examination of table 3 reveals that sucrose enters the body fluids studied very readily. In the case of edema fluid, considerable concentrations were present as early as ten to fifteen minutes following the injection. It remained within these body fluids for considerable

TABLE 2

Rate of disappearance of sucrose from the blood stream in the control group following intravenous injection

INTERVAL FOLLOWING INJECTION		BLOOD SERUM LEVEL	INTERVAL FOLLOWING INJECTION		BLOOD SERUM LEVEL
Hours	Minutes	Mgm. per cent	Hours	Minutes	Mgm. per cent
	2	320	18		4
	5	312	18		1
	5	258	21		2
	15	200	20		1.5
	35	228	24		0.7
2	45	104	24		1.5
4	50	45	24		1.5
6	15	44	48		none
8		17	48		none
11		9			

periods of time, these fluids apparently serving as a reservoir for sucrose and maintaining a relatively high level in the blood for much longer periods than in normal individuals. Initially the sucrose in the blood is much higher than in the body fluid, followed by a period of approximately equal concentration, the blood level finally tending to fall below that of the fluid.

DISCUSSION. Contrary to many statements in the literature, our results indicate that sucrose diffuses rapidly into body fluids. We realize it is possible that in cases with abnormal fluid accumulations, the capillaries may have greater than normal permeability. Nevertheless, it is in just such patients that sucrose is often used clinically as a diuretic and hence any supposed advantage in its use in such instances cannot be based on lack of tissue penetration. To us, it seems unlikely that sucrose would fail to penetrate into extracellular fluids even in normal individuals since sucrose is a relatively small, non-electrolyte molecule. As previously stated, there is experimental evidence in the literature to support its diffusibility into extracellular fluids.

In our experiments, we did not attempt to evaluate the clinical advantages of sucrose as compared with other sugars.   The amounts of sucrose we injected

TABLE 3

Distribution of sucrose in patients with abnormal accumulations of extracellular fluids

NO.	CLINICAL DIAGNOSIS	FLUIDS STUDIED	INTERVAL AFTER INJECTION		BLOOD SERUM CONCENTRATION	CONCENTRATION IN FLUID
			Hours	Minutes	Mgm. per cent	Mgm. per cent
1	Cirrhosis of liver	Abdominal	1½		211	72
			24		52	60
			51		20	27
			97		9	9
2	Cardiac failure, hydrothorax	Pleural	24		8	25
3	Cirrhosis of liver	Abdominal	20		2	8
4	Cardiac failure, hydrothorax	Pleural	3		7	35
5	Pleurisy with effusion (tuberculous)	Pleural	24		1	4
6	Cirrhosis of liver	Abdominal	23		6	25
7	Cirrhosis of liver, hypoproteinemia	Edema fluid		35		12
			1			18
			2			31
			3½			36
			5			36
			7			38
			24		8	10
8	Cirrhosis of liver, hypoproteinemia	Edema fluid		15		16
				45		31
			1¼			44
			1¾			55
			2¼			59
			2¾			59
			3¼			48
			4¼		31	
		Abdominal	24		2	5
9	Chronic nephritis with edema	Edema fluid		10		12
				25		19
				45		24
			1			31
			1	20		33
			1	45		29

are smaller than those usually employed for therapeutic effect and no attempt was made to estimate the amount of diuresis, fall in intracranial pressure or

other clinical effect. Our results should not be interpreted to contradict the many articles in the literature wherein the authors claim a superiority for hypertonic sucrose solutions as compared with those of glucose. However, we do believe our experiments indicate that claims to such superiority should not be based upon lack of diffusibility into extracellular fluid.

SUMMARY

1. An accurate method for the determination of sucrose is presented. Sterile samples are incubated with cultures of Bacillus coli communior and Bacillus coli communis and sucrose determined by appropriate modification of Roe's method for fructose. Since the former organism ferments sucrose while the latter does not, the difference in the two samples represents sucrose.

2. Sucrose was injected intravenously into patients who had no evidence of renal or circulatory failure and without abnormal fluid accumulations, and its rate of disappearance from the blood stream was studied. Only traces were present after twenty-four hours and the serum contained no sucrose after forty-eight hours.

3. In patients with abnormal extacellular fluid accumulations, sucrose was found to diffuse rapidly into ascitic, pleural and edema fluids, following intravenous injection. These fluids were found to act as reservoirs for sucrose, so that it was retained in the tissues and remained at relatively high levels in the blood for long periods of time.

REFERENCES

(1) KEITH, N. M., M. H. POWER, AND R. D. PETERSON. This Journal 109: 62, 1934.
(2) LAVIETES, P. H., J. BOURDILLON AND K. A. KLINGHOFFER. J. Clin. Investigation 15: 261, 1936.
(3) HUBBARD, R. S. AND K. TERPLAN. Arch. Path. 20: 307, 1935.
(4) ANDERSON, R. K. AND R. S. HUBBARD. Arch. Path. 30: 981, 1940.
(5) ROE, J. H. J. Biol. Chem. 107: 15, 1934.
(6) SOMOGYI, M. J. Biol. Chem. 86: 655, 1930.
(7) KEITH, N. M. AND M. H. POWER. This Journal 120: 203, 1937.

THE INFLUENCE OF THE ACCELERATOR NERVES ON THE BASAL HEART RATE OF THE DOG[1]

QUILL MURPHY

From the Department of Physiology, University of Wisconsin Medical School, Madison

Most investigators have believed that the cardio-accelerator fibers are in a state of tonic activity. Hunt (1) working with anesthetized dogs found that section of the accelerator nerves to the heart seldom failed to cause slowing. In experiments cited on three dogs, the heart rates before cutting the accelerator nerves were 237, 219 and 174 beats per minute. After cutting the accelerator nerves, these heart rates fell respectively to 159, 138 and 159 beats per minute. Hunt noted this decrease in the heart rate following section of the cardio-accelerator fibers regardless of the drug used as the anesthetic agent and when anesthesia was produced by section of the crura cerebri or by compression of the cerebrum.

Bronk and his collaborators (2, 3, 4) studied the activity of the cardiac sympathetic center by recording the action potentials in the cardiac-nerves from the stellate ganglion of the cat. He found a continuous discharge of impulses from these accelerator nerves which he interpreted as evidence for tonic activity of the accelerator nerves.

Gasser and Meek (5) studied the effect of removal of the stellate ganglia on the heart rate of trained dogs at rest. In this instance the normal pulse rates of the dogs at rest were determined by making them lie quietly until the pulse rates no longer decreased. The stellate ganglia were removed and after the dogs recovered their resting heart rates were again determined. The resting heart rates of the six dogs reported ranged from 78 to 140 beats per minute before removal of the stellates. After the removal of the stellates, the range of the resting heart rates was from 66 to 80 beats per minutes. This decrease in the resting heart rate after the removal of the stellates suggested accelerator tone.

In the present work dogs were trained until their resting heart rates reached a value which was low and consistent on consecutive determinations and which was considered the basal resting rate. The effect of cardiac sympathectomy on this basal rate was then investigated.

METHODS AND RESULTS. To establish the basal heart rates normal dogs were trained by gentle methods to lie quietly on a table for a period of 60 minutes each day. The dogs were not allowed to eat any food during the 12 hour period preceding each rest period. Extraneous noises were reduced to a minimum and the apprehension of the dogs was also reduced as much as possible. The animals did not go to sleep while on the table. Pulse rates were taken frequently by palpation from the femoral artery until the dogs became accustomed to the procedure. Some of the dogs were accustomed to the electrocardiograph elec-

trodes and to the noise made by the machine so that heart rates could be obtained by watching the beam of the electrocardiograph and electrocardiograms could be taken to verify the heart rates obtained by palpation. Since sinus arrhythmia frequently accompanied slow heart rates, the pulse was counted for 60 seconds each time the heart rate was determined.

Resting heart rates were obtained on 24 dogs. In table 1 it can be seen that the average heart rate before conditioning the dogs was 96 beats per minute.

TABLE 1

Resting heart rates of dogs at near basal conditions and the effect of cardiac sympathectomy on such rates

| DOG NO. | HEART RATE BEFORE CONDITIONING | USUAL RANGE OF HEART RATE AT NEAR BASAL CONDITIONS | | TIME OF LAST OBSERVATION AFTER SYMPATHECTOMY | HEART RATE OF LAST OBSERVATION AFTER SYMPATHECTOMY |
		Before sympathectomy	After sympathectomy		
	beats/min.	beats/min.	beats/min.	days	beats/min.
1	100	56–65	60–65	70	72
2	95	42–50	42–50	24	45
3	95	42–55	42–55	70	48
4	95	45–55	48–55	34	48
5	90	44–50	54–60	55	60
6	110	55–65	60–65	21	64
7	96	46–65	54–65	21	58
8	90	43–55	42–55	35	55
9	112	52–60	48–60	12	58
10	96	44–50	48–50	11	48
11	95	57–65			
12	80	55–60			
13	85	55–60			
14	90	44–55			
15	95	50–55			
16	95	44–50			
17	130	44–50			
18	120	45–50			
19	85	55–60			
20	90	45–50			
21	96	45–50			
22	85	50–60			
23	80	48–55			
24	90	50–60			
Average.......	96	50–56			

This rate was obtained on the first day of conditioning after the animal had lain quietly for at least 10 minutes. Also recorded in table 1 is the usual range of the heart rates of each of the dogs at near basal conditions. The lowest rate in this range was obtained in some dogs after a very few days and on several occasions. However, with other dogs a period of several weeks of conditioning was necessary before such a rate could be obtained, and even then it might be reached only once during the period of conditioning. After the basal heart rate was reached it seldom varied on consecutive determinations by more than 10 beats

per minute by the end of the 60 minute rest period. Twelve of the twenty-four dogs had a resting rate of 45 beats per minute or less on at least one day while the basal rates were being determined, and no rate failed to slow below 58 beats per minute during the period of conditioning.

In 10 of the trained dogs the stellate ganglia and sympathetic chains with the upper five thoracic ganglia were removed. The surgical procedure was completed in a single stage in all of the dogs except dog 3. In this animal a two stage removal was performed with the second stage following the first by a week.

After the dogs recovered the resting heart rates were again observed. The resting rates on the first few days following the sympathectomy were considerably above the basal level, but only the dog from which the sympathetics were removed in two stages failed to return within 14 days approximately to the heart rates before sympathectomy recorded in table 1. Six of the dogs returned to the normal basal rate within 7 days. In dog 5 there was an increase of 10 beats per minute in the resting heart rate after the sympathectomy Dog 7 showed an increase of 8 beats per minute after the sympathectomy. In the remaining dogs there was only a difference of 5 beats per minute or less from that established as the basal resting rate before the operation. Thus no significant difference between the basal heart rates before and after the removal of the accelerator nerves was found although 8 of the dogs were tested for 21 days or more following the sympathectomy.

This is evidence opposed to the general idea that the accelerators are in a constant state of tonic activity.

DISCUSSION. Since the present data show that the heart rate of the normal dog at rest may be brought into a range of 42 to 65 beats per minute, the results of Hunt should not be indicative of accelerator tonus in a normal resting dog. Under the conditions of his experiment there is no doubt that the accelerator center in his dogs was in a state of activity, in some cases almost maximum activity, as evidenced by the very high heart rates he obtained before the cardiac sympathetics were cut. Even after cutting the cardiac sympathetics the heart rates Hunt obtained were considerably above those found for the normal resting, unanesthetized dog as given in the present report.

Gasser and Meek did not bring their dogs to a basal level, meaning the lowest rate that could be attained by training. Undoubtedly, however, the care subsequent to the operation brought their animals somewhat nearer such a level, as is indicated by the uniformity in the pulse rates after the removal of the stellate ganglia. If a true resting rate was not attained before the operation, any approach to it after the removal of the stellates would naturally have been attributed by these authors as evidence of the loss of accelerator tone.

Bronk and his collaborators (2, 3, 4) have shown that in nembutalized cats there is a more or less constant flow of impulses out over the cardiac sympathetics. However, these impulses were reduced or abolished by conditions which increase vagal tone such as adrenalin injection or increased pressure in the carotid sinus. This would indicate that as the cardio-inhibitory center was stimulated the accelerator center was reciprocally inhibited.

These results of Bronk's are not inconsistent with the present observations.

Under ordinary conditions the heart is partly held at a higher rate by reduced vagal tone. Evidence for this may be found in the results of atropine injection which at once raises the basal resting rate, and in the motility of the heart rate in exercised animals deprived of their sympathetics as shown by Gasser and Meek.

In view of past work and the present data it would seem that the accelerators in the intact animal are concerned with adapting the heart rate to any of the constantly occurring changes in bodily conditions. During the ordinary waking hours both the vagal and accelerator centers are being constantly bombarded by impulses from various sense organs and psychical regions of the brain. Thus the heart rate is constantly maintained above its possible basal rate both because of reduced vagal tone and increased accelerator tone. However, when the animal is shielded from these effects of sensation and emotion to a considerable extent as is done when a basal heart rate is reached, the accelerator mechanism ceases its activity and the heart rate slows to a rate determined largely or entirely by vagal tone. For this basal rate it does not matter whether the accelerator mechanism is in existence or not. The accelerator mechanism would therefore seem to be truly an emergency mechanism.

If this conception holds it would be the function of the vagus to determine a true basal resting rate. In a trained animal lying comfortably in a noiseless room there would be nothing to throw the accelerator mechanism into activity either by direct sensory impulses or by a reflex secretion of adrenalin sufficient to affect the heart directly. In this resting condition the accelerators do not seem to be in tonic activity, otherwise the basal resting rate before and after cardiac sympathectomy could not be identical.

SUMMARY

The near basal heart rates of normal unapprehensive dogs that had been without food for 12 hours and had rested quietly for 60 minutes ranged from 50 to 56 beats per minute.

Bilateral removal of the stellate and upper 5 thoracic ganglia failed to result in an appreciable change in this basal heart rate.

REFERENCES

(1) HUNT, R. This Journal 2: 395, 1899.
(2) BRONK, D. W. AND L. K. FERGUSON. Proc. Soc. Exper. Biol. and Med. 30: 339, 1932.
(3) BRONK, D. W., L. K. FERGUSON AND D. Y. SOLANDT. Proc. Soc. Exper. Biol. and Med. 31: 579, 1934.
(4) BRONK, D. W., L. K. FERGUSON, R. MARGARIA AND D. Y. SOLANDT. This Journal 117: 237, 1936.
(5) GASSER, H. S. AND W. J. MEEK. This Journal 34: 48, 1914.

THE EFFECTS OF A DIET DEFICIENT IN THE VITAMIN B COMPLEX ON SEDENTARY MEN

E. EGAÑA, R. E. JOHNSON, R. BLOOMFIELD, L. BROUHA, A. P. MEIKLEJOHN, J. WHITTENBERGER, R. C. DARLING, C. HEATH, A. GRAYBIEL AND F. CONSOLAZIO

From The Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, Department of Medicine, Harvard Medical School, and The Fatigue Laboratory, Harvard University

In a general way, we know the subsistence requirements of the essential food-stuffs, but it is uncertain what amounts are necessary for the highest efficiency, and little is known about what effects the sudden withdrawal of any of the dietary essentials may have upon a man's capacity to carry on his usual work. The present experiments are a contribution to one aspect of this problem. Sedentary subjects lived on a diet grossly deficient in the vitamin B complex. The course of the deficiency was followed by the amount of thiamine excreted in the urine, since this reflects closely and rapidly the dietary intake of thiamine (23). In addition most students of experimental B deficiencies have stressed the constant, early appearance of easy fatigue as a symptom (15, 29). Therefore, particular attention was paid to the reactions of our subjects to easy work in the shape of walking uphill and to exhausting work in the shape of running uphill on a motor-driven treadmill.

Our results show clearly that under the dietary regime described below, all subjects become deficient at least in thiamine within three or four weeks, that there is measurable physical deterioration in this time, and that brewers' yeast is a complete and adequate supplement to the deficient diet that we used.

EXPERIMENTAL PROCEDURE. For clarity in the rest of the paper the various periods in the experiment on each subject will be called, in order, the *normal period before*, lasting about one week, in which the subject ate his usual diet; the *deficient period*, lasting from three to four weeks, during which he ate the diet deficient in the vitamin B complex; the *yeast period*, lasting one or two weeks, in which he continued the deficient diet, but took brewers' yeast every day; and the *normal period after*, in which he reverted to his usual diet. The periods followed each other without interruption, and the subject carried on his usual laboratory and hospital duties all the time. The measurements described in detail below were made in each period.

The seven subjects were healthy physicians between 27 and 42 years of age. None was a trained athlete, and all were leading the sedentary life customary among laboratory workers. They were allowed unlimited amounts of unfortified white bread, soda crackers, butter, cheddar type cheese, macaroni, spaghetti, polished rice, farina, heavy cream, sugar, honey, molasses, tapioca, salad oil, egg white, ice cream, puffed rice, coffee, tea, salt, pepper, vinegar, hard candies, and gelatine. Each day they had small amounts of orange juice,

grape juice, onions, and lettuce; and twice a week, very small portions of meat, fish or poultry.

The caloric intake was not restricted, the subjects being urged to try to keep their weight constant. The average intake was from 2500 to 3000 calories daily. Every attempt was made to keep the protein intake adequate, especially by consumption of cheese. In addition, the subjects took daily doses of halibut liver oil[1] containing 8500 I.U. of vitamin A and 1700 I.U. of vitamin D, and 50 mgm. of ascorbic acid.[1] By present standards and tables (4, 5) the diet was adequate in riboflavin, certainly deficient in thiamine, and presumably deficient in nicotinic acid, pantothenic acid and pyridoxine. However, data on these last three are unsatisfactory, nor are subsistence requirements for them well known. Therefore, the present paper deals mainly with the specific problem of thiamine deficiency.

At the end of the deficient period, each subject began taking thirty-six grams per day of Standard Brands' brewers' yeast type 2019,[1] for which the analysis is:

Thiamine	200–300 micrograms per gram
Riboflavin	70 micrograms per gram
Nicotinic acid	600 micrograms per gram
Pantothenic acid	200 micrograms per gram
Pyridoxine	85 micrograms per gram
Protein	45 grams per 100 grams

A. *Observations on the subject at rest.* a. Samples of urine were collected for 24 hours frequently. Their thiamine content was estimated by the method of Egaña and Meiklejohn (7). b. At the end of the normal period before and at the end of the deficient period, thiamine saturation tests were made after the method of Robinson, Melnick and Field (23). The subject took 5 mgm. of thiamine hydrochloride by mouth and then collected two successive twelve-hour specimens of urine. This type of saturation test is indicative of deficiency when only a small percentage of the dose of thiamine is excreted. c. The urinary nitrogen excretion of three subjects was measured throughout all four periods in order to estimate the protein intake. Routine estimations were made for all subjects of plasma protein, erythrocytes, leukocytes, hemoglobin, cell volume, and differential count. d. Basal observations. His basal metabolic rate was estimated by the open circuit gasometer method. Urine was collected for estimating his basal nitrogen, lactate and pyruvate excretion. Venous blood was taken for estimation of sugar, lactate and pyruvate. e. Weight was measured at frequent intervals. f. Complete urinalyses were made on all urine samples. g. Careful watch was kept for dermatological signs. h. Electrocardiograms were taken in each period. i. For three subjects, vibration sense was tested quantitatively with the pallesthesiometer. j. For two subjects, estimations were made of the free thiamine and diphosphothiamine in blood cells and plasma.

B. *Observations on responses to exercise.* The routine followed in these observations was changed in detail but not in principle after the first four subjects had been studied. We merely made more different observations on the last group of three subjects. The response to exercise was measured on a motor-driven treadmill as previously described (22), walking first for 15 minutes at 3.5 m.p.h. and then running to exhaustion at 7 m.p.h. on an 8.6 per cent grade. No subject was able to run longer than four and a half minutes. The measurements made during the work were ventilation, oxygen consumption, carbon dioxide excretion, and pulse rate. As soon as he was exhausted, the subject sat on a stool and his oxygen debt was estimated for ten minutes in three periods of 1, 2 and 7 minutes. At 5 minutes after the run, capillary blood was drawn for estimation of sugar and lactate, and blood pressure was taken at intervals. After this period of 10 minutes, the subject lay on a bed and his oxygen debt was measured up to one hour's recovery. Pulse rates were taken manually at intervals and venous blood was drawn at 15, 30, 45 and 60 minutes for estimation of sugar, lactate, and pyruvate. At the end of this period a sample of urine was obtained covering the whole period of exercise and recovery. Lactate, pyruvate and nitrogen were all estimated in these samples, and a complete urinalysis was made.

The various analytical procedures were: Gasometric analyses by standard methods; lactate by the method of Edwards (6); pyruvate by the method of Lu (18) with the additional precaution of stabilizing the pyruvate by iodoacetate after Bueding and Wortis (3); blood sugar by the method of Folin and Malmros (9); urinary nitrogen by the method of Keys (17); blood diphosphothiamine by the method of Goodhart and Sinclair (10).

RESULTS. Out of the very large number of observations, there were some positive findings and many negative ones. No single subject showed all of the changes described below, but a majority did. In general, a single subject would show either no change in a particular function, or else he would react like the rest of the group, never showing the reverse of what other subjects did.

A. *Dietary observations.* Table 1 lists the changes in one subject in weight, daily urinary nitrogen excretion, and daily thiamine excretion in the different periods. Conclusions to be drawn from these data for all subjects are: (1) Caloric intake was adequate in three cases; the maximal weight loss in any of the other subjects was 8 pounds. (2) Protein intake was adequate, at least 60–80 grams per day as calculated from the urinary nitrogen, the proteins consumed being mostly first-class proteins. (3) Thiamine deficiency was definite within four weeks, as measured by daily thiamine excretion (see fig. 1 and table 1) and by the thiamine saturation tests which are summarized in table 2. It is seen that the percentage of the test dose excreted in 24 hours was invariably much smaller in the deficient period than in the normal period before. (4) Administration of yeast was followed by restoration to very high levels of the excretion of thiamine daily.

B. *Observations on the subject at rest.* a. In agreement with the observations of other students of early B complex deficiency (15, 29), the symptoms of our

subjects were somewhat ill defined.   A general feeling of lack of well being was noticed by five subjects, easy fatigue by five, loss of efficiency in the daily work

TABLE 1

Significant positive and negative findings in sedentary subjects

	SUBJECT	Normal before	Deficient Day 7	Deficient Day 14	Deficient Day 21	Yeast Day 3	Yeast Day 10	Normal after	TYPE OF MEASUREMENT
Weight (kilos)	R. D.	92.6	92.3	91.5	91.3	90.7	91.3	92.5	Nutritional state
Nitrogen excretion (grams in 24 hours)	R. D.	15.7	11.1	10.0	10.0	11.2	15.0	15.3	
Thiamine excretion (micrograms/24 hrs.)	R. D.	64	17	10	0	250	730	200	
BMR (calories/sq.m. and hr.)	J. W.	35.2	36.3	35.8	34.4	36.6	36.3	38.7	Basal data
Non-protein RQ	J. W.	0.85	0.81	0.79	0.76	0.80	0.84	0.85	
% of BMR due to carbohydrate	J. W.	37	31	25	16	26	39	40	
Blood lactate (mg. %)	J. W.	9	8	7	8	7	11	11	
Blood pyruvate (mg. %)	R. D.	0.6	1.0	1.0	1.0	1.6	1.2	1.0	
Ventilation (cc./kilo and min.)	R. D.	670	680	700	720	690	680	670	Walking uphill
Oxygen consumption (cc./kilo and min.)	R. D.	27.7	27.0	28.0	28.8	28.0	26.4	26.7	
Maximal heart rate	R. D.	167	161	164	165	173	165	163	
Maximal O₂ consumption	J. W.	41.7	45.3	43.8	44.4	45.2	43.6	45.0	Running uphill
Maximal CO₂ output	J. W.	55.5	57.4	54.0	54.6	55.2	55.0	55.2	
Maximal heart rate	J. W.	200	193	195	194	194	198	200	
Maximal blood sugar (mgm. %)	R. J.	156	137	126	128	124	132	134	Recovery after running
Maximal blood lactate (mgm. %)	J. W.	145	142	100	127	133	136	125	
Maximal blood pyruvate (mgm. %)	R. D.	4.9	5.0	4.6	4.9	5.1	4.7	4.9	
Oxygen debt in 1 hour (cc./kilo)	J. W.	169	161	174	157	149	157	162	

Note: In the table header, "Deficient" spans Days 7, 14, 21 and "Yeast" spans Days 3, 10, all under the overall heading "PERIOD".

TABLE 2

Thiamine saturation tests

Each subject took 5 mgm. thiamine hydrochloride by· mouth and then collected two successive samples of urine covering twelve hours each.

SUBJECT	THIAMINE EXCRETION IN 24 HOURS AFTER TEST DOSE Normal before	THIAMINE EXCRETION IN 24 HOURS AFTER TEST DOSE Deficient
	micrograms	*micrograms*
Br	789	127
Da	795	109
Eg	746	241
He	365	118
Jo	460	97
Wh	708	116

by five, sleepiness, lethargy and lack of ambition by three, forgetfulness by two, constipation by one, poor appetite by two, irritability by two, paresthesias by two, gastro-intestinal upsets in the shape of slight nausea or diarrhea by two,

and muscle and joint pains during motion by one. The sum total of these symptoms is not striking; although none of the subjects felt perfectly well, none of them suffered acutely. After beginning to take yeast the subjects recovered relatively slowly and three subjects felt worse for a few days than at any time previously. By the end of the yeast period all felt perfectly well, even though they continued to eat the deficient diet. It was characteristic that certain subjects did not realize that they had been in poor condition until they improved after taking yeast. *b.* At the end of the deficient period, two subjects had notice-
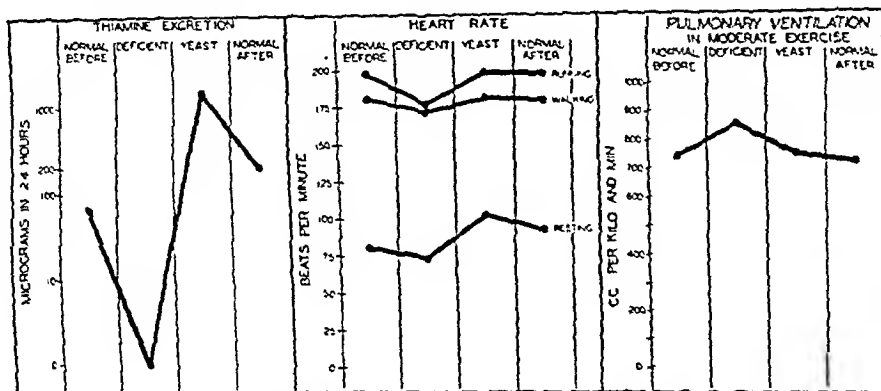
Fig. 1. Urinary excretion of thiamine, heart rate at rest and during activity, and pulmonary ventilation during moderate exertion, of sedentary subjects before, during and after deficiency in the vitamin B complex.

TABLE 3

Free thiamine and diphosphothiamine in the blood of subject unit

PERIODS	THIAMINE INTAKE	THIAMINE EXCRETION IN URINE	CELL VOLUME	DIPHOSPHO-THIAMINE IN CELLS	FREE THIAMINE IN CELLS	FREE THIAMINE IN PLASMA
	microgms. per day	*microgms. per day*	*per cent*	*microgms. per 100 ml. whole blood*	*microgms. per 100 ml. whole blood*	*microgms. per 100 ml. whole blood*
Normal before..........	1,000	85	39.2	7.2	2.4	1.8
Deficient...............	190	6	40.7	3.0	1.0	Trace
Yeast.................	10,171	1,354	41.0	3.5	2.7	0.7
Normal after...........	2,000	201	42.0	10.0	2.5	1.6

able cheilosis and scaling at the angles of the nose; these cleared up within a few days after taking the yeast. No subject had any signs within the mouth. *c.* No abnormality was observed in the three subjects on whom quantitative measurements of vibratory sense were made by the pallesthesiometer. *d.* The changes in thiamine and diphosphothiamine in the blood of subject *W* are shown in table 3. By the end of the deficient period when his urinary thiamine excretion was only 6 micrograms per day, the diphosphothiamine and the free thiamine in the cells and the free thiamine in the plasma had reached low levels. Even after 10 days of yeast, these values were not back to normal, except for the free

thiamine in the cells.   Only after 10 days of normal diet were they all back to normal.   This slow return corresponds to the slow disappearance of symptoms exhibited by this subject.   e. The urine remained normal with respect to volume, specific gravity, albumin, sugar acetone bodies, and formed elements.   f. There were no significant changes in the plasma proteins, erythrocytes, leukocytes, hemoglobin, cell volume or differential counts, with the exception of a tendency in two subjects to a slight normochromic normocytic anemia.

Electrocardiograms (leads 1, 2, 3, and 4-F) were taken on all subjects before, during and after the test period.   Considerable care was taken to ensure uniformity of procedure.   The tracings were always taken after a rest period with the subject recumbent and usually under strictly basal conditions.   The electrocardiograph was tested for accuracy, the standardization recorded on each lead strip, and the same chest area was always used in taking lead 4-F.   A minute analysis was made of the amplitude, form and duration of the several waves and intervals including the Q-T interval according to the formula:

$$Q - T = K \sqrt{\frac{\text{cycle}}{\text{length}}}$$

In 5 out of the 7 cases there were no significant differences in any of the electrocardiographic measurements; in the remaining 2 there were alterations in the T waves which require special comment.   In one subject, the electrocardiogram taken before the test period shows a lower amplitude of the T waves which require special comment.   In one subject, the electrocardiogram taken before the test period shows a lower amplitude of the T waves in leads 1 and 2 than at any time during the test period of one month or afterward.   It was concluded that the vitamin deficiency which the subjects developed had no appreciable effect on the electrocardiogram.   In one other subject there was slight but progressive lowering of the T waves in the limb leads during the test period. When yeast was added to the diet the T waves returned to their normal amplitude.   Thus, in 7 subjects who were on a diet deficient in vitamin B, for periods up to one month, only one had electrocardiographic changes and these were of a minor character.

The experience of various investigators has not been uniform with respect to the electrocardiographic changes in patients with beriberi or in subjects with induced vitamin B-1 deficiency.   Aalsmeer and Wenckebach (1) and Hashimoto (11), who have studied patients in the Orient with beriberi, have emphasized the fact that the electrocardiograms are rarely abnormal even in the presence of marked cardiac insufficiency.   There were occasional exceptions to this rule and Hashimoto had described a case of acute pernicious beriberi in which negative T waves in lead 1 of the electrocradiogram became upright 50 hours after the administration of vitamin B-1.   Minor changes in the electrocardiogram including sinus tachycardia, right axis deviation, and slight alterations in amplitude of QRS and T waves.

Significant changes in the electrocardiogram on the other hand have often been observed in patients with occidental beriberi (usually multiple vitamin de-

ficiency) (5). Kepler (16), in his oft-quoted case report, states that the electrocardiogram was normal, but inspection of the record reveals sagging of the RS-T segments in leads 1 and 2 and abnormally low amplitude of the T waves in all leads. Scott and Hermann (24) observed low voltage, inverted T waves and other abnormalities in the electrocardiograms from patients with beriberi and suggested that even in mild cases definite myocardial changes may occur. Weiss and Wilkins (28) observed abnormalities in the electrocardiograms from all but 5 of 57 patients with vitamin B deficiency but with no clinical evidence of heart disease. However many of the electrocardiographic alterations were of a minor character and most of their patients were in an age group when electrocardiographic abnormalities are frequently seen.

Observations on induced vitamin B-1 deficiency in man have shown (29) that although characteristic signs and symptoms develop they do not resemble beriberi in any of its typical forms. Only minor alterations in the electrocardiogram have been observed consisting chiefly in a diminution of the amplitude of the T waves. These alterations do not occur in all subjects and usually appear only after long periods of deficiency. They rapidly disappear after the administration of vitamin B-1. Individual variations, age of the subject, initial state of nutrition, the degree of activity, temperature of the environment, and nutritional deficiency other than vitamin B-1 are some of the variables which may account for the inconsistent results.

Our own experience leads us to conclude that in healthy young subjects, vitamin B (chiefly B-1) deficiency for short periods rarely causes significant changes in the electrocardiogram. The electrocardiographic alterations observed in patients with beriberi, especially in the Occident, may be due to prolonged nutritional deficiency, often in old patients with some degree of underlying heart disease. The rare occurrence of electrocardiographic abnormalities in patients with Oriental beriberi is difficult to explain, but may be due in part to the acute onset of the disease often in young and otherwise healthy subjects.

Basal functions (table 1 and fig. 1). *a.* The BMR decreased in the subjects who lost weight, and did not change in those who maintained their weight. The nonprotein RQ decreased steadily in two of the three subjects on whom it was measured most carefully, and increased again in the yeast period. The interpretation of this is obscure. It is the opposite of what is usually seen when a diet high in carbohydrate is eaten. *b.* The basal pulse rate decreased in four subjects who lost weight, and did not change in two who maintained weight. This is in agreement with the observations of Benedict et al. (2) on the effects of simple inanition. It is not the typical bradycardia of beriberi heart disease (28). The basal blood pressure was not changed. *c.* Basal blood sugar, blood lactate, blood pyruvate, and urinary lactate and pyruvate were not changed. These observations lend weight to the view that these measurements are of little diagnostic use in early thiamine deficiency, since changes in them are small and inconstant (25, 26, 29), in contrast to changes late in the course of thiamine deficiency in animals (8, 27), and in man (20).

C. Observations on the response to exercise. 1. Response to moderate work

(walking uphill). *a.* Functional cardiovascular alterations appeared in a majority of the subjects. In contrast to the tachycardia of beriberi and other types of thiamine deficiency, our subjects showed, during the deficient period, either their normal rise of the pulse rate during walking, or else an abnormally small rise. The pulse rate in recovery after walking likewise tended to be abnormally slow. These changes were reversed in the yeast period. The blood pressure showed no significant changes in any period or subject. *b.* The blood lactate and blood sugar showed no significant changes from the normal. *c.* The oxygen consumption and carbon dioxide excretion during this moderate work tended in most cases to increase slightly as deficiency progressed. The mechanical efficiency, measured by oxygen consumption, therefore was impaired by the deficiency (see table 1). This impairment was reversed when yeast was added to the deficient diet.

2. Response to exhausting work (running uphill). *a.* The duration of this run is an important measure of physical fitness. In the deficient period it was abnormally short in two of the three fittest subjects. More significant than this change is the fact that five of the six subjects were able to run longer in the yeast period than in either of the normal periods. *b.* The response of the blood pressure remained normal. The pulse rates showed significant alterations in the deficient period. In contrast to the tachycardia of beriberi (1, 28) our subjects tended to have an abnormally low maximal pulse, or else their usual response (table 1 and fig. 1). Even more significant was the fact that all subjects during the deficient period were abnormally slow in attaining their maximal heart rate during the run. These changes were all reversed in the yeast period. *c.* The maximal oxygen consumption and maximal carbon dioxide output remained the same in all periods (table 1). This suggests that no early defect in the oxidation-reduction systems of the muscles is to be found, and that the decarboxylating mechanisms remain unaffected in early deficiency. *d.* In contrast to animals (8) and man (12, 20) in late severe thiamine deficiency, our subjects had abnormally low maximal blood lactates, blood sugars, and urinary lactate and pyruvate excretions when deficient. Blood pyruvate was not affected.

3. Recovery after exhausting exercise. *a.* The blood pressure showed inconstant variations from subject to subject, and period to period. The pulse rates were either normal or slightly slower. *b.* There were neither significant changes in the total oxygen debt, measured for one hour, and its rate of repayment, nor in the total carbon dioxide excretion and its rate of excretion. *c.* The rates of removal of excess lactic acid, pyruvic acid and sugar from the blood stream, calculated according to the equation of Margaria and Edwards (19), were unaffected by the deficiency, although the maximal levels were lower in the deficient period. This finding contrasts markedly with what is seen in human beriberi (12, 21) and in animals (8).

4. Quantitative variations in physical fitness before, during, and after deficiency. *a.* In moderate work: As deficiency progressed, moderate exercise tended to cause an abnormally high pulmonary ventilation. These changes are shown in detail for one subject exposed at intervals to moderate work in the

shape of running on the level at 7.0 m.p.h. (fig. 1). This increased ventilation is perhaps associated with the easy fatigue noticed by the subjects, and may perhaps be regarded as incipient dyspnea on exertion. There tended to be a progressive decrease in mechanical efficiency in easy work as described above. This decrease was reversed by addition of yeast to the diet. *b*. In exhausting work: A quantitative index of fitness for hard exertion has been described by Johnson, Brouha and Darling (13). When applied to our subjects, it shows that in the deficient period there was impairment of fitness in four subjects and that all subjects improved in the yeast period (table 3). In fact, most of them were fitter in the yeast period than in the first or second normal periods. *c*. In repeated exhausting work: One of the most significant symptoms, noted especially by the two fittest subjects, was that after a single bout of exhausting exercise in the deficient period, subjective recovery before a second bout one

TABLE 4

Physical fitness for hard work

(Expressed as index of fitness according to Johnson, Brouha and Darling, poor being below 40; good, above 75)

SUBJECT	NORMAL BEFORE	DEFICIENT	YEAST	NORMAL AFTER
Jo*				
First run......................	63	60	6S	74
Second run, 1 hour later...........		6S	S5	
Wh*				
First run......................	47	56	5S	50
Second run, 1 hour later...........		45	5S	
He.................................	3S	33	37	32
Bl.................................	37	30	45	37
Da*................................	22	21	26	2S
Eg.................................	17	17	24	20

* These subjects exercised at least twice a week throughout the experiment.

hour later, and actual performance of the second bout, were abnormally poor. This phenomenon was measured and the fitness indices for the first and second exhausting runs in each period are shown in table 4. It is seen that the first run was better in the yeast period than in the deficient period; the second run was much better in the yeast period than in the deficient period; in comparison to the first run, the second was much better in the yeast than in the deficient period. It is worth mentioning that these measurable improvements agreed very well with the subjects' own feelings in the two periods. The practical conclusions may be drawn that B complex deficiency leads to diminution of fitness for a single bout of exhausting work; to an even more marked unfitness for repeated exhausting work, and therefore that the processes of recuperation after exercise are impaired by the deficiency.

DISCUSSION. Early deficiency of the vitamin B complex in our subjects was accompanied within four weeks by regular changes only in the following three

ways: 1. The urinary excretion of thiamine dropped to low levels, and the percentage of thiamine excreted after a test dose was lowered. 2. Most of the subjects had mild symptoms. 3. There was moderate deterioration in physical fitness characterized by an abnormal increase in the pulmonary ventilation during moderate work, by a decreased ability to withstand exhausting work and especially by a decreased capacity for repeated exhausting work, reflecting clearly a lack of adequate recuperation.

All of the other measurements both cardiovascular and metabolic, showed no regular or constant alteration from period to period. In particular there were no characteristic changes in the electrocardiogram, in the blood pressure, blood lactate, blood pyruvate, blood sugar, urine lactate, or urine pyruvate at rest, during or after exercise. In other words, none of the metabolic and cardiovascular signs found in *late deficiency* can be relied upon to detect *early deficiency*. In general, it can be said that symptoms and signs relieved by yeast are very likely due to previous deficiency in the B complex.

The moderate deterioration in our sedentary subjects is strikingly different from what is seen in manual laborers exposed to a diet deficient in the B complex, who suffer within 5 days serious impairment of their fitness for sustained hard work and complain of acute symptoms (14).

SUMMARY

1. Seven healthy physicians subsisted on a diet deficient in the vitamin B complex, but adequate in calories and in proteins, for periods up to four weeks, then added brewers' yeast to this diet for two weeks, and finally reverted to a normal diet.

2. Deficiency, at least of thiamine, within 4 weeks was demonstrated by analysis of the daily thiamine output and by the rate of excretion of test doses of thiamine.

3. The symptoms were mild and vague, the most constant being easy fatigue, loss of ambition and loss of efficiency in daily work.

4. There was moderate deterioration of the subjects' physical fitness for exhausting exercise, and, particularly, poor recuperation between repeated bouts of exhausting exercise.

5. The above changes were the only regular findings in the deficiency, and they were all reversed by addition of brewers' yeast to the diet.

6. All other metabolic measurements showed slight abnormal changes or none at all in rest, moderate exercise, exhausting exercise and after exhausting exercise. These measurements were oxygen consumption, carbon dioxide excretion, blood lactate, blood pyruvate, blood sugar, urine lactate and urine pyruvate.

7. The cardiovascular changes were inconstant. There was never a tachycardia on exertion. On the contrary, the subjects tended to have abnormally slow heart rates in moderate exercise and in exhausting exercise. There were no abnormal changes in the blood pressure. In only one subject did the electrocardiograph show significant changes.

8. It is emphasized that under the conditions of these experiments only the

amounts of vitamins found in the urine, and symptoms and signs suggesting deterioration of the efficiency of the whole organism can be relied on to detect early deficiency.

9. Symptoms and signs that are cleared up by administration of brewers' yeast in adequate amounts are suggestive of deficiency in the B complex.

10. These findings on sedentary subjects are contrasted with rapid and striking effects of vitamin B complex deficiency in men doing daily hard work.

REFERENCES

(1) AALSMEER, W. C. AND K. F. WENCKEBACH. Arch. f. inn. Med. **16**: 193, 1929.
(2) BENEDICT, F. G., W. R. MILES, P. ROTH AND H. M. SMITH. Human vitality and efficiency under prolonged restricted diet. Carnegic Inst. of Washington, 1919.
(3) BUEDING, E. AND H. WORTIS. J. Biol. Chem. **133**: 585, 1940.
(4) COWGILL, C. Vitamin B requirements in man. Yale Univ. Press, 1934.
(5) EDDY, W. H. AND G. DALLDORF. The avitaminoses. Williams & Williams, 1941.
(6) EDWARDS, H. T. J. Biol. Chem. **125**: 571, 1938.
(7) EGAÑA, E. AND A. P. MEIKLEJOHN. J. Biol. Chem. **141**: 859, 1941.
(8) FISHER, R. B. Biochem. J. **25**: 1410, 1931.
(9) FOLIN, O. AND H. MALMROS. J. Biol. Chem. **83**: 115, 1929.
(10) GOODHART, R. S. AND H. M. SINCLAIR. Biochem. J. **33**: 1099, 1939.
(11) HASHIMOTO, H. Am. Heart J. **13**: 580, 1937.
(12) INAWASHIRO, R. AND E. HAYASAKA. Tohokû J. Exper. Med. **12**: 1, 1928.
(13) JOHNSON, R. E., L. BROUHA AND R. C. DARLING. Rev. Canadienne de Biol. In press.
(14) JOHNSON, R. E., R. C. DARLING, W. H. FORBES, L. BROUHA, E. EGAÑA AND A. GRAYBIEL. J. Nutrition, 1942. In press.
(15) JOLLIFFE, N. Symposium on vitamins. Chicago Univ. Press 1942.
(16) KEPLER, E. J. J. A. M. A. **85**: 409, 1925.
(17) KEYS, A. J. Biol. Chem. **132**: 181, 1940.
(18) LU, G. D. Biochem. J. **33**: 249, 1939.
(19) MARGARIA, R. AND H. T. EDWARDS. This Journal **107**: 681, 1934.
(20) PLATT, B. S. AND G. D. LU. Quart. J. Med. **5**: 35, 1936.
(21) PLATT, B. S. AND G. D. LU. Biochem. J. **33**: 1538, 1939.
(22) ROBINSON, S. Arbeitsphysiol. **10**: 251, 1938.
(23) ROBINSON, W. D., D. MELNICK AND H. FIELD, JR. J. Clin. Investigation **19**: 399, 1940.
(24) SCOTT, L. C. AND G. R. HERREMANN. J. A. M. A. **90**: 2083, 1928.
(25) SHILS, M. E., H. G. DAY AND E. V. MCCOLLUM. Am. J. Med. Sci. **201**: 561, 1941.
(26) TAYLOR, F. H. L., S. WEISS AND R. W. WILKINS. J. Clin. Investigation **16**: 833, 1937.
(27) THOMPSON, R. H. S. AND R. E. JOHNSON. Biochem. J. **29**: 694, 1935.
(28) WEISS, S. AND R. W. WILKINS. Ann. Int. Med. **11**: 104, 1937.
(29) WILLIAMS, R. D., H. L. MASON, R. M. WILDER AND B. F. SMITH. Arch. Int. Med. **66**: 785, 1940.
(30) WILLIAMS, R. D. AND T. D. SPIES. Vitamin B₁. Macmillan, 1938.

THE ACID-BASE EQUILIBRIUM OF THE BLOOD IN EXERCISE

E. S. TURRELL AND S. ROBINSON

From the Department of Physiology, Indiana University Medical School, Bloomington

An increased blood lactate in humans during exercise is accompanied by a decrease in base bound as bicarbonate consequently causing a decrease in the CO_2 combining capacity of the blood. The reaction

$$BHCO_3 + \text{Lactic acid} \rightarrow B(\text{lactate}) + H_2CO_3$$
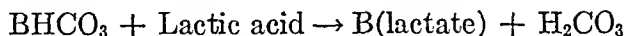
and the associated extra output of CO_2 through the lungs acts as one of the principal buffering mechanisms of the body. However observations upon the relation of the magnitude of changes in lactate and CO_2 capacity have been contradictory. Mellanby and Thomas (1920) and Evans (1922), by addition of lactic acid to drawn blood, found that the decline in CO_2 content was less than the increase in blood lactate. Results of similar experiments performed in this laboratory have shown close agreement yet the picture is not identical with that seen in blood drawn after exercise. In six observations on blood drawn after exercise, Barr, Himwich and Green (1923) obtained wide variations and found a greater change in blood lactate than in CO_2 capacity in only two cases. Dill, Talbott and Edwards (1930) found in general a greater decline in CO_2 capacity of the blood. Dennig et al. (1931) found approximately equal changes when the blood lactate rose to 10 mEq. per liter. Robinson and Harmon (1941) found that the decreases in CO_2 capacity, at physiologically high concentrations of blood lactate, were smaller than the corresponding increases in lactate.

By further study of this problem we have attempted to relate changes in blood lactate, CO_2 capacity, and serum pH and to determine the rôle of the various mechanisms in buffering acid as it is accumulated during exercise. Samples of blood were drawn from human subjects in the basal state and after exercise on the same day. Various intensities of exercise, which consisted of running on a motor driven treadmill or in competitive races, were used to produce different concentrations of blood lactic acid in the men. For comparison of changes in pH, CO_2 capacity, lactate, and related changes in available base, arterial blood samples were drawn under oil and treated with heparin. Several samples of venous blood were used in the comparison of the variations in lactate concentration with those of CO_2 capacity. Blood lactate was determined by the method of Edwards (1938), and plasma protein by micro-Kjeldahl analysis. HbO_2 and CO_2 capacity were determined by equilibration of blood with O_2 and CO_2 pressures of 200 and 40 mm. Hg respectively at 37°C. as described by Dill in Henderson's book (1928). Analyses of blood samples for both content and capacity of HbO_2 and CO_2 were done on the Van Slyke apparatus. The pH values of arterial blood samples were calculated by means of the Henderson-Hasselbalch equation and some over the entire range of values

obtained were checked by the electrometric method as described by Dill, Daly and Forbes (1937). The two methods checked each other very closely. The term "CO₂ capacity" as used here is defined as the CO_2 content of oxygenated whole blood at 37°C. and 40 mm. Hg CO_2 tension.

RESULTS. Figure 1 reveals a distinct relationship between the increase in lactate (Δ lactate) and the decrease in CO_2 capacity (ΔCO_2 capacity). Increases of lactate up to 4 mEq. per liter are accompanied by approximately equivalent decreases in CO_2 capacity. In this range almost all of the base used in neutralization of the acid is obtained from base bound as bicarbonate. However, as the concentration of base bound as bicarbonate is decreased beyond this
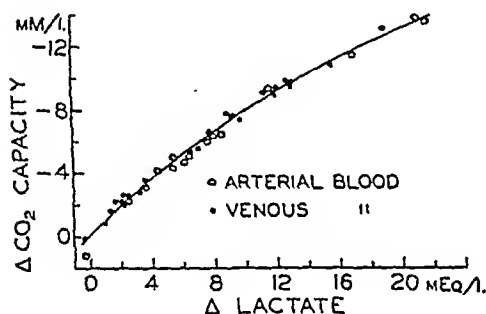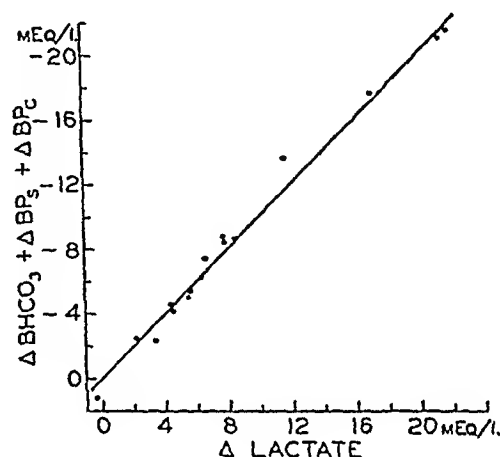
Fig. 1. Increases above the basal level of blood lactate (Δ lactate) in relation to corresponding decreases in CO_2 capacity (ΔCO_2 capacity) of the blood of men after various intensities of exercise.

Fig. 2. Increases above the basal level of blood lactate (Δ lactate) plotted against corresponding decreases of available base from bicarbonate (Δ BHCO₃), serum proteins (Δ BP$_s$) and cell proteins (Δ BP$_c$) in arterial blood drawn after various intensities of exercise. The straight line indicates equal changes of lactate and base.

point by higher lactate concentrations the ratio of $\dfrac{\Delta\ CO_2\ \text{capacity}}{\Delta\ \text{lactate}}$ becomes progressively smaller. With the accumulation of lactic acid the pH of the blood decreases and approaches the isoelectric point of the blood proteins decreasing their base binding capacity and releasing base for neutralizing the acid. Thus as the concentration of lactic acid becomes higher the plasma proteins and hemoglobin account for a greater fraction of the base used to neutralize the accumulated acid.

Calculations of changes in the arterial blood samples have revealed the relation of increases in lactate concentration to decreases in available base from bicarbonate, cell proteins, and plasma proteins. Decreases in base bound as bicarbonate in the whole blood (Δ BHCO₃)ᵦ were calculated from values of the

CO_2 tension and content.   Base contributed by plasma proteins (ΔBP_s) has been calculated from the data of Van Slyke, Hastings, Hiller and Sendroy (1928).

$$BP_s = 0.104 \text{ (gram protein) (pH}_s - 5.08)$$

Grams of plasma proteins per liter of blood were calculated from protein analysis of plasma and hematocrit determination of plasma and cell volumes.   Similarly the decrease in base bound by protein in the cells (ΔBP_c) has been calcu-

TABLE 1

Arterial blood changes in exercise

	ΔLACTATE MEQ./l.	ΔCO_2 CAPACITY MM./l.	ΔPH$_8$	$\Delta(BHCO_3)_b$ MEQ./l.	ΔBP_c MEQ./l.	ΔBP_8 MEQ./l.
1	-0.3	1.2	0.03	1.0	0.14	0.12
2	2.2	-2.1	-0.03	-2.3	-0.13	-0.04
3	3.5	-3.1	-0.07	-1.8	-0.25	-0.21
4	4.5	-4.1	-0.05	-3.5	-0.48	-0.17
5	5.5	-4.3	-0.09	-3.8	-0.83	-0.32
6	5.5	-5.0	-0.11	-4.3	-0.68	-0.37
7	6.2	-4.6	-0.11	-4.8	-0.91	-0.37
8	6.5	-5.1	-0.07	-6.5	-0.72	-0.24
9	7.7	-6.1	-0.11	-7.1	-1.30	-0.48
10	7.8	-6.4	-0.07	-6.7	-1.25	-0.46
11	8.5	-6.5	-0.14	-6.7	-1.50	-0.41
12	11.6	-9.0	-0.18	-11.0	-1.86	-0.71
13	17.0	-11.5	-0.30	-13.1	-3.23	-1.12
14	21.1	-13.6	-0.38	-15.3	-4.05	-1.49
15	21.6	-13.5	-0.40	-15.5	-4.14	-1.70
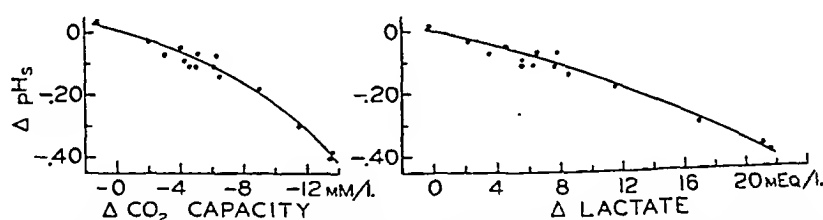
Fig. 3. The relationship of increases above the basal level of blood lactate (Δ lactate) and decreases in CO_2 capacity (Δ CO_2 capacity) to the decline of pH$_s$ (Δ pH$_s$) of arterial blood after various intensities of exercise.

lated from the formula for oxygenated cells derived by Dill, Edwards and Consolazio (1937).

$$BP_c = HbO_2[-0.5 \text{ (pH}_c)^2 + 10.625pH_c - 48.46]$$

In table 1 have been tabulated the changes found in 15 arterial blood samples drawn after exercise.   The increases in blood lactic acid (Δ lactate) are about equivalent to corresponding combined decreases in available base according to the equation

$$\Delta \text{ lactate} = \Delta(BHCO_3)_b + \Delta BP_s + \Delta BP_c$$

This relationship is represented in figure 2.

Figure 3 shows changes in the pH of arterial blood serum (ΔpH_s) as related to Δ lactate and Δ CO_2 capacity. Throughout the range of lactate concentrations studied the ratio of $\dfrac{\Delta pH_s}{\Delta \text{ lactate}}$ is almost constant being only slightly greater at high values of lactate. In contrast the ratio of $\dfrac{\Delta pH_s}{\Delta CO_2 \text{ capacity}}$ steadily increases as the CO_2 capacity is lowered. This again demonstrates the decreased buffering action of bicarbonate as the concentration is lowered.

The maximum ΔpH_s of -0.40 pH units was a decrease, measured in a well-trained athlete, from a basal pH_s value of 7.37 to a pH_s of 6.97 after work. Bock, Field and Adair (1923) and others have measured similarly low pH_s values in diabetic coma. It has been our experience that lactate values of 22 mEq. per liter are not uncommon in trained athletes after hard races. In such cases the arterial pH_s probably drops to about 7.0 yet these athletes show no ill effects aside from a breathlessness after the race from which they soon recover.

SUMMARY

Blood samples drawn from human subjects in the basal state and after various intensities of exercise were analyzed for O_2 and CO_2 capacity, lactic acid, plasma proteins, and serum pH. Comparison of increases in lactate concentration (Δ lactate) and decreases in CO_2 capacity (Δ CO_2 capacity) up to 4 mEq. per liter showed approximately equivalent changes. Beyond this point decreases in CO_2 capacity became progressively smaller than corresponding increases in lactate concentration. Base for neutralization of the lactic acid at low concentrations was obtained principally from bicarbonate. At the higher lactate concentrations hemoglobin and plasma proteins accounted for an increased fraction of this base. Decreases in serum pH of arterial blood (ΔpH_s) varied directly with Δ lactate and Δ CO_2 capacity. The maximum blood lactate value measured was 22 mEq. per liter with a corresponding serum pH of 6.97.

REFERENCES

BARR, D. P., H. H. HIMWICH AND R. P. GREEN. J. Biol. Chem. **55**: 495, 1923.
BOCK, A. V., H. FIELD, JR. AND G. S. ADAIR. J. Metab. Res. **4**: 27, 1923.
DENNIG, H., J. H. TALBOTT, H. T. EDWARDS AND D. B. DILL. J. Clin. Investigation **9**: 60, 1930.
DILL, D. B., C. DALY AND W. H. FORBES. J. Biol. Chem. **117**: 569, 1937.
DILL, D. B., H. T. EDWARDS AND W. V. CONSOLAZIO. J. Biol. Chem. **118**: 635, 1937.
DILL, D. B., J. H. TALBOTT AND H. T. EDWARDS. J. Physiol. **69**: 267, 1930.
EDWARDS, H. T. J. Biol. Chem. **125**: 571, 1938.
EVANS, C. L. J. Physiol. **56**: 146, 1922.
HENDERSON, L. J. Blood, a study in general physiology. New Haven, Yale University Press, 1928.
MELLANBY, J. AND C. J. THOMAS. J. Physiol. **54**: 178, 1920.
ROBINSON, S. AND P. M. HARMON. This Journal **132**: 757, 1941.
VAN SLYKE, D. D., A. B. HASTINGS, A. HILLER AND J. SENDROY, JR. J. Biol. Chem. **79**: 769, 1928.

SEXUAL BEHAVIOR IN RATS WITH LESIONS IN THE ANTERIOR HYPOTHALAMUS

GEORGE CLARK

From the Department of Anatomy, Medical College of the State of South Carolina, Charleston

In 1938 Fisher, Magoun and Ranson (1) reported that cats with small electrolytic lesions in the anterior hypothalamus failed to come into heat and that if such lesions were placed in pregnant cats abnormal deliveries or failure to deliver occurred. These results led the writer to test the sexual behavior of a group of rats which had been prepared for a study in diabetes insipidus (2). The findings in these rats were later amplified by the preparation of another series. In all of these animals electrolytic lesions were placed in the anterior hypothalamus with the aid of the Horsley-Clarke stereotaxic instrument (3) as modified for the rat (4). No changes in sexual behavior were noted in those rats with bilateral lesions in the lateral half of the anterior hypothalamus. In some of the rats with medially placed lesions, however, there was found constant vaginal estrus with refusal to mate while a few others exhibited constant vaginal diestrus. Only a few of the rats studied were males but, although the lesions were quite minute, an occasional rat refused to mate. The location of the lesions and the behavior of some of these rats were apparently quite similar to those of the male and female guinea pigs reported by Dey et al. (5, 6, 7, 8, 9, 10).

In all of the rats with electrolytic lesions which showed sexual abnormalities the damage included portions of the medial half of the anterior hypothalamus but many with lesions in this region seemed quite normal and it proved to be impossible to correlate symptoms with lesions. Since the possibility existed that the sexual abnormalities found could be explained on the basis of pituitary damage even though this gland were far removed from the anatomical limits of the lesion (Ingram, (11) has suggested a similar explanation for disturbances in carbohydrate metabolism following hypothalamic lesions in cats) it was decided to attempt the destruction of portions of the anteromedial hypothalamus by some other method which might minimize this possible damage.

The following procedure was finally adopted. Using a small dental crown saw, a small cut was made in the calvarium just caudad of the coronal suture. Through this opening a knife was plunged at a predetermined angle to the base of the brain. The first knife used was prepared from a thin dental spatula 3 mm. wide with a rectangular end. Later a 4 mm. instrument was made from a thin razor blade and a wire guide was used to insure the proper angle. In most cases the plane of section passed caudad of the anterior commissure and reached the base of the brain between the middle of the optic chiasma and the anterior border of the median eminence. The high mortality (over 50 per cent) perhaps precludes the use of this method in other animals.

The first five males and all the females were allowed to recuperate from the

immediate effects of the operation and were then placed with animals of the opposite sex in large cages. Daily vaginal smears were made on the females until a pregnancy sign was found or until the animals were sacrificed while the males were observed at irregular intervals. After recovery from the immediate effects of the operation the remaining thirteen males, which were not sexually mature at the time of operation, were placed with females of the same age in small cages (1 male and 1 female per cage). Those males which sired normal litters were sacrificed while the remainder (10) were rotated between large cages with four females and small cages with one female. All these normal females were smeared daily. Two males were sacrificed when plugs or sperm were found in the vaginal smears of the females they were with. This left eight rats which had been with from 10 to 13 different normal females during 20 to 30 periods of estrus. For a terminal experiment eight normal females whose AM smear consisted only of round nucleated cells were chosen. Beginning at 11:30 a.m. these were tested for heat at hourly intervals by placing a normal male with them. After the first female came into heat (3:30 p.m.) the remaining eight experimental males were rotated repeatedly through the cages containing the females in heat. There were two cages with three females and one cage with two females. Each male was left with a group of females for ten minutes. At 6:30 p.m. all the females were in heat (i.e., had accepted normal males). There was only one male, a markedly obese animal, which did not make the preliminary investigations such as are made by normal males—smelling and licking the external genitalia of the females, ruffing the hair of the shoulder region, etc. This was done, however, in a rather cursory manner and they did not show the intense excitement such as is usually seen in normal males. In an attempt to increase the sexual excitement of the females and thus, possibly, that of the experimental males, normal males were placed with the females and allowed to copulate once or twice. This procedure markedly increased the excitement of the females but affected only one male. The exception, a slightly stunted individual, which previously had shown only perfunctory interest in the females began mounting and copulating. For the remainder of the test period this rat's activity was almost identical with that of a normal male. By 9 p.m. when the experiment was concluded each of the males had had at least 30 chances (times with females times the number of females per cage) to mate.

There were only five females in the series. All of these ran normal sexual cycles and four became pregnant and delivered litters. Only one of the four suckled her young, however. Since the behavior of the other three seemed comparable to that of the occasional young female which refuses to care for her first litter these three were bred several times but they repeatedly refused to suckle their young. These three were sacrificed three days after the delivery of their final litters. Their mammary glands were fixed after the manner of Jeffers (12) and their glands were compared with those of a normal female which was sacrificed three days after delivery and whose litter was killed when born. Histologically there was no difference between the glands of the normal and the experimental animals. Sperm and plugs were repeatedly found in the vaginal

smears of the one rat which failed to become pregnant, but neither pregnancy nor pseudopregnancy resulted. Despite repeated attempts (sometimes two males of proved potency were kept in the cage with this female) no changes in the length of the cycle occurred.

All the animals were killed by decapitation, the brains were removed and fixed in formalin, the reproductive organs were fixed in Bouin's and all the tissues were embedded in paraffin. A few of the brains were sectioned transversely, in these it was difficult to determine if the lesion reached the base of the brain; others were sectioned sagittally, in these the lateral limits were hard to define; the remainder and most satisfactory were sectioned horizontally. All were mounted serially and stained with cresyl violet.

A study of the brains of these rats, in contrast to that of the animals with electrolytic lesions, was quite instructive. If one considers first those animals which showed definite abnormalities of sexual behavior one must be guided by the
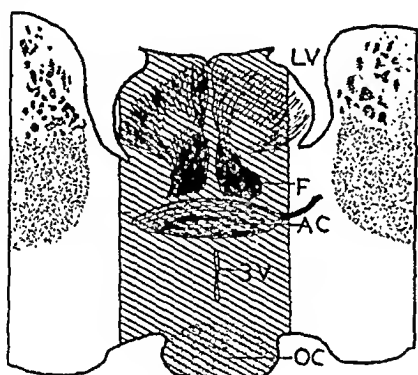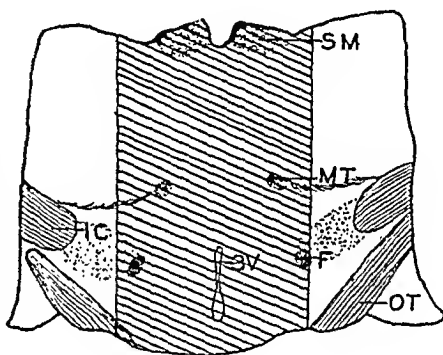
Fig. 1   Fig. 2

Figs. 1 and 2. Diagrammatic reconstructions of the most rostral (fig. 1) and the most caudal (fig. 2) lesions in the group of rats with symmetrical lesions and normal sexual behavior. Abbreviations as follows: *AC*, anterior commissure; *IC*, internal capsule; *F*, fornix; *LV*, lateral ventricle; *MT*, mammilothalamic tract; *OC*, optic chiasma; *OT*, optic tract; *SM*, stria medullaris; *3V*, third ventricle.

principle that the smallest lesion which abolishes a given set of responses is the most important. Unilateral damage failed to abolish sexual behavior but several males whose lesions barely crossed the midline failed to copulate. This may perhaps indicate that some structure concerned in the integration of sexual behavior may lie adjacent to the third ventricle. However we occasionally find in our colony a male or female rat, otherwise normal, which refuses to copulate or to become pregnant. If one approaches the problem from the other angle, that is, if one studies only those rats which showed normal or approximately normal sexual behavior then one may discard all animals with asymmetrical lesions and one does not have the normal variation in sexual drive to consider. There were ten rats in this group, 8 males and 2 females. The lesions of two of these are shown diagrammatically in figures 1 and 2. In making these drawings the horizontal sections were projected upon appropriate transverse sections from the brain of a normal rat.

These two lesions are the most rostral (fig. 1) and the most caudal (fig. 2) of the group. Laterally they extend beyond the fornices but their exact ventral extent was difficult to determine and it is possible that at least some of the fibers occupying a very superficial position on the base of the brain may have escaped destruction. The lesions in the remaining eight animals were quite similar in extent and lay at intervals between the two shown in the figures. It should be emphasized that the behavior of these rats could not be predicted from the lesions. Two rats, one of which failed to mate and another which seemed to be quite normal, might have almost identical lesions.

Several conclusions may be drawn from these findings. First, there seemed to be a tendency for damage to the medial half of the anterior hypothalamus to depress sexual activity. Second, transverse lesions extending from fornix to fornix and lying at various levels from the middle of the optic chiasma to the anterior border of the median eminence are not incompatible with normal sexual behavior. Third, if there is in this region any area or areas essential for normal sexual behavior, fibers to and from this "center" must pass by one of two routes: 1, directly ventrad and pursue a very superficial course therefrom; and 2, directly laterad to beyond the fornices. Fourth, if there is any such structure its connections must be rather diffuse as it is probable that in at least one of these rats this "center" must have been bisected.

This work, then, neither affirms nor denies the possibility that there may exist in the medial half of the anterior hypothalamus a structure or structures essential for the integration of normal sexual behavior. It does indicate the improbability that such is the case and very definitely limits the course of fibers to and from this hypothetical center.

REFERENCES

(1) FISHER, C., H. W. MAGOUN AND S. W. RANSON. Am. J. Obst. and Gynec. **36**: 1, 1938.
(2) CLARK, G. Quart. Bull., Northwestern Univ. Med. School **14**: 96, 1940.
(3) RANSON, S. W. Psychiat. en neurol. bl. **38**: 534, 1934.
(4) CLARK, G. Science **90**: 92, 1939.
(5) DEY, F. L., C. FISHER, C. M. BERRY AND S. W. RANSON. This Journal **129**: 39, 1940.
(6) BROOKHART, J. M., F. L. DEY AND S. W. RANSON. Proc. Soc. Exper. Biol. **44**: 61, 1940.
(7) DEY, F. L., C. FISHER AND S. W. RANSON. Am. J. Obst. and Gynec. **42**: 459, 1941.
(8) DEY, F. L. Am. J. Anat. **69**: 61, 1941.
(9) BROOKHART, J. M., F. L. DEY AND S. W. RANSON. Endocrinology **28**: 561, 1941.
(10) BROOKHART, J. M. AND F. L. DEY. This Journal **133**: 551, 1941.
(11) INGRAM, W. R. (In discussion of paper by C. N. H. LONG. Research Nerv. and Ment. Dis. Proc. (1939) **20**: 498, 1940).
(12) JEFFERS, K. R. Am. J. Anat. **56**: 257, 1935.

RADIOACTIVE PHOSPHORUS STUDIES ON STRIATED AND CARDIAC MUSCLE METABOLISM[1]

JACOB SACKS AND CHARLES H. ALTSHULER

From the Department of Pharmacology, University of Michigan Medical School, Ann Arbor

It has been demonstrated by the use of radioactive phosphorus (P^{32}) that the formation of lactic acid in contraction of striated muscle does not involve the interchanges of phosphate groups postulated by the currently accepted mechanism for glycolysis (8). For the isotope technique to yield results of critical value, it is essential that the resting metabolism of the muscles lead to a differential distribution of the P^{32} among the organic compounds present. Fortunately, the conditions chosen did result in such a differential distribution.

The present work was undertaken primarily to study the time course of the distribution of P^{32} in the resting metabolism of muscle, and thereby to determine the basis for the differential effects previously found. The data so obtained might be expected to indicate whether the postulated phosphorylating glycolysis may be operating in the resting metabolism of the intact cell, even though it does not function in the activity metabolism. Observations were also made on the distribution of P^{32} in the intact beating heart, for comparison with the data of Barker, Furchgott and Shorr (2) on quiescent heart slices.

Experiments were carried out on frogs and cats. The general procedure was to inject the P^{32}, in the form of Na_2HPO_4, into the ventral lymph sac in frogs, and subcutaneously in cats, then, after the lapse of one or more hours, to freeze the tissues in situ. The various P compounds were separated from trichloroacetic acid filtrates of the powdered tissues, the P converted to $MgNH_4PO_4$, and measurements of the P^{32} content per millimeter P were then made. Data were obtained on striated muscle of frogs at 1, 2, 24 and 48 hours after injection of the P^{32}, and on striated muscle, cardiac muscle, and plasma of cats at 1, 2, 4 and 24 hours after injection. Determinations of P^{32} content were made on inorganic P, phosphocreatine (PC), the two readily hydrolyzable groups of adenosine triphosphate (ATP), the difficultly hydrolyzable one of the adenylic acid residue (AA), the hexosemonophosphate (HMP) of striated muscle and heart, and on the plasma inorganic P.

The frogs were anesthetized with urethane for the short periods of observation; for the longer ones, they were decerebrated a day in advance. Immediately after the injection of the P^{32} they were placed in small wire cages in running water; at the proper time cage and contents were dropped into the freezing mixture. The muscles of both hind legs were taken together.

The cats were anesthetized with pentobarbital just prior to the injection of the P^{32}. In the 24-hour group, the anesthesia was administered 2 hours before the tissues were to be sampled. Since the previous work (8) had shown that

---

a tetanic contraction does not alter the P^{32} content of the PC or ATP, it was felt that the small amount of muscular activity shown by cats confined to cages would not vitiate the results. The gastrocnemius muscles were prepared for freezing in the usual way, the trachea was cannulated, and one carotid artery exposed for cannulation. At the proper time the muscles were frozen, the artery cannulated, and blood run directly into a flask containing crystalline heparin. The thorax and pericardial sac were then opened under artificial ventilation, and the freezing mixture poured over the heart. The time elapsed from freezing the first muscle to freezing the heart was between 4 and 6 minutes.

The phosphorus compounds were separated from the trichloroacetic acid filtrates by the methods previously described (8). The procedure for the isolation of the P present in the different forms was modified somewhat. One portion of the filtrate was treated with magnesia mixture made up with magnesium and ammonium nitrates, precipitating the inorganic phosphate; the PC in the filtrate from this was hydrolyzed by adding nitric acid to 2 N, excess ammonium molybdate, and letting stand $1\frac{1}{2}$ hours at room temperature. Another portion of the trichloroacetic acid filtrate was treated with barium hydroxide for the separation and isolation of ATP and HMP. The Ba precipitate, containing inorganic phosphate, ATP and adsorbed PC, was dissolved in 2N nitric acid, ammonium nitrate added to a concentration of 5 per cent, and excess ammonium molybdate added. This was let stand $1\frac{1}{2}$ hours at room temperature to precipitate inorganic phosphate and that liberated by the hydrolysis of the adsorbed PC; this precipitate was discarded, and the filtrate heated 20 minutes in a boiling water bath to separate the two readily hydrolyzable groups of the ATP. The phosphate group of the AA was separated by heating the filtrate from the previous precipitation on the steam bath for 24 hours. The HMP was separated from the barium hydroxide filtrate. This was made approximately 1 N with sulfuric acid, the precipitated $BaSO_4$ removed by centrifugation, and the supernatant let stand over night to hydrolyze the PC. This phosphate was precipitated by magnesia mixture, and the HMP in the filtrate broken down by wet ashing with sulfuric and nitric acids. The inorganic phosphate formed was then precipitated with magnesia mixture. Plasma inorganic phosphate was precipitated by magnesia mixture from trichloroacetic acid filtrates.

All the phosphate precipitates were converted to $MgNH_4PO_4$, washed well, and dissolved in dilute nitric acid. Small aliquots were taken for determination of P, and the major aliquots transferred to 5 ml. beakers (those used with the Beckman pH meter) and evaporated to dryness. Determinations of relative P^{32} content were made with a Geiger-Müller counter, using a glass bubble type of Geiger-Müller tube with a background count of 10 per minute. Control observations showed that the errors due to non-uniform distribution of the evaporation residue on the bottom of the beaker were negligible. The activity of the experimental samples ranged from 2 or 3 net counts per minute up to several hundred. The counting period was at least 8 minutes for even the most active samples; for those of low activity the counting period was sufficiently prolonged to give a minimum of 200 net counts above background. The meas-

urements on these samples are probably accurate to within 10 per cent; on the the active samples the accuracy of the counting is between 2 and 3 per cent.

The quantities of P^{32} injected, per kilogram body weight of the animals, were such as corresponded to between 5×10^5 and 2×10^6 counts per minute with the Geiger-Müller tube used. The total amounts of phosphorus injected were naturally subject to considerable variation. In the tables of data, all measurements have been corrected for decay to a standard time for each animal, and have been recalculated to the basis of 1×10^6 counts per minute per kilogram body weight. Thus the data on any two animals are directly comparable.

The data on the cat muscles are given in table 1. Comparison of the P^{32} contents of the PC and ATP with that of plasma inorganic phosphate shows that the rate of exchange of these compounds with plasma phosphate is quite low. At 2 hours after the injection the apparent turnover is only 1 part in 200. The turnover rate of the ATP is somewhat lower than that of the PC. The turnover rate of the AA is about one-fourth that of the two readily hydrolyzable groups of the ATP.

The HMP fraction shows a peculiar behavior in that 2 hours after the injection, the P^{32} content of this fraction is three times as high as in the PC or ATP, but at 4 hours it has dropped to a small fraction of this value while the PC and ATP have increased their P^{32} contents slightly. At 24 hours the relation between the HMP and the PC and ATP is essentially the same as at 4 hours. These findings can be explained on the basis that, in addition to the expected metabolic turnover of HMP, a temporary accumulation of this substance takes place on the cell membrane when a large amount of phosphate is injected. The material on the membrane is hydrolyzed, with liberation of the phosphate into the extracellular phase, as the phosphate is excreted. The metabolic turnover should be independent of the absolute amount of phosphate injected, whereas the accumulation on the membrane might be expected to depend on this absolute amount.

To test this hypothesis, the 2-hour experiments were repeated with a sample of P^{32} which had been subjected to prolonged intensive bombardment, so that an adequate amount of radioactivity was contained in a fraction of a milligram of P, instead of in the much larger amounts that had previously been used. The results, which are given in table 2, are those which would have been anticipated from the above hypothesis. The P^{32} contents of plasma inorganic phosphate, the PC and the ATP are the same as when the large amount of phosphate was given, but the P^{32} content of the HMP is well below that of the other two organic compounds, so that it is comparable to the 4-hour experiments. These data show that the turnover rates of the PC and ATP are independent of the absolute amount of phosphate injected, and also show the HMP does not exchange with other intracellular components.

Turning now to the organic compounds of the heart muscle, in table 3, it is obvious that the turnover rate with plasma phosphate is very much greater than in resting striated muscle. At 2 hours, for example, the P^{32} contents of the PC and ATP are over 20 times as great as in the striated muscle. The HMP

shows a lower P^{32} content than the other two compounds. The tremendous difference between heart and striated muscle is not due to the constant activity

TABLE 1

Distribution of radioactive phosphorus (P^{32}) in resting muscles of cats

Values are in terms of net counts per minute per milligram P, calculated to the basis of 1×10^6 counts per minute injected, per kilogram body weight.

PLASMA INORG. P	MUSCLE INORG. P	CALC. INTRA-CELLULAR INORG. P	PHOS-PHO-CREA-TINE	ADENO-SINETRI-PHOS-PHATE	HEXOSE MONO-PHOS-PHATE	ADENYLIC ACID	PLASMA INORG. P	MUSCLE INORG. P	TOTAL P INJECTED
							mgm. per cent	*mgm. per cent*	*mgm. per kgm.*

A. 1 hour after injection

PLASMA INORG. P	MUSCLE INORG. P	CALC. INTRA-CELLULAR INORG. P	PHOS-PHO-CREA-TINE	ADENO-SINETRI-PHOS-PHATE	HEXOSE MONO-PHOS-PHATE	ADENYLIC ACID	PLASMA INORG. P	MUSCLE INORG. P	TOTAL P INJECTED
17,100	303	*	34	48	40		6.5	25	9.7
14,050	395	36	34	49	17		5.6	24	9.8
15,500	415	*	21	22			6.3	16	9.0
7,800	260	*	16	10	26		7.9	15	9.1
Av.....13,612	343		26	32	28				

B. 2 hours after injection

PLASMA INORG. P	MUSCLE INORG. P	CALC. INTRA-CELLULAR INORG. P	PHOS-PHO-CREA-TINE	ADENO-SINETRI-PHOS-PHATE	HEXOSE MONO-PHOS-PHATE	ADENYLIC ACID	PLASMA INORG. P	MUSCLE INORG. P	TOTAL P INJECTED
15,300	905	172	93	99	216		6.9	16	2.8
18,800	540	*	48	56	308		7.6	25	2.8
15,400	610	21	89	57	390	18	5.2	15	6.3
15,100	690	190	115	100	146	13	5.5	18	6.3
Av.....16,150	686		86	78	265	16			

C. 4 hours after injection

PLASMA INORG. P	MUSCLE INORG. P	CALC. INTRA-CELLULAR INORG. P	PHOS-PHO-CREA-TINE	ADENO-SINETRI-PHOS-PHATE	HEXOSE MONO-PHOS-PHATE	ADENYLIC ACID	PLASMA INORG. P	MUSCLE INORG. P	TOTAL P INJECTED
5,300	420	218	95	82	43	30	7.2	20	8.5
7,150	224	37	63	50	28	18	6.0	25	8.5
15,000	485	29	142	94	30	17	5.0	18	2.8
11,250	442	110	175	113	27	26	4.9	21	2.9
Av..... 9,675	393	99	119	85	32	23			

D. 24 hours after injection

PLASMA INORG. P	MUSCLE INORG. P	CALC. INTRA-CELLULAR INORG. P	PHOS-PHO-CREA-TINE	ADENO-SINETRI-PHOS-PHATE	HEXOSE MONO-PHOS-PHATE	ADENYLIC ACID	PLASMA INORG. P	MUSCLE INORG. P	TOTAL P INJECTED
547	169	159	126	117	57	39	5.2	22	11.2
915	154	132	114	101	37	18	6.0	22	11.2
1,055	153	120	125	121	51	15	5.6	17	8.5
1,115	126	93	99	67	60	11	5.6	19	8.5
690	188	169	145	157	58	52	5.6	17	9.1
735	248	230	191	181	120	50	5.6	16	9.1
Av..... 843	173	151	133	124	64	31			

* P^{32} content of extracellular fluid P less than that of plasma P.

of the former, for the data of Barker, Furchgott and Shorr (2) on quiescent heart slices show a very close correspondence to the present findings. They found that

at equilibrium the PC and ATP had about one-sixth the P^{32} content of the inorganic phosphate of the medium, and that of the HMP was considerably lower. The 2-hour data here show practically this 1 to 6 ratio between the PC and ATP and plasma inorganic phosphate. The logical interpretation of this similarity of findings on the beating heart and the non-beating slices is that the contraction process in this organ, as in striated muscle, does not involve the phosphate interchanges of the glycolytic cycle.

Comparison of the data on frog muscles, in table 4, with those of the cat muscles shows that the apparent turnover rate and time course of the phosphate distribution are essentially the same in the two species. The data on the frogs 48 hours after injection emphasize that the HMP does not interchange with PC,

TABLE 2

Influence of absolute amount of P injected on distribution of P^{32} in resting muscles of cats

Values are in terms of net counts per minute per milligram P, calculated to the basis of 1×10^6 counts per minute injected, per kilogram body weight.

A. 2 hours after injection of large amount of P

P INJECTED	INORGANIC P	PHOSPHOCREATINE	ADENOSINE TRIPHOSPHATE	HEXOSEMONO- PHOSPHATE	PLASMA INORG. P
mgm. per kgm.					
2.8	905	93	99	216	15,300
2.8	540	48	56	308	18,800
6.3	610	89	57	390	15,400
6.3	690	115	100	146	15,100
Average.......	686	86	78	265	16,150

B. 2 hours after injection of small amount of P

P INJECTED	INORGANIC P	PHOSPHOCREATINE	ADENOSINE TRIPHOSPHATE	HEXOSEMONO- PHOSPHATE	PLASMA INORG. P
0.25	620	45	58	17	19,900
0.25	790	86	74	29	11,700
0.31	870	97	78	39	13,500
0.31	680	71	95	38	12,500
Average.......	740	75	76	31	14,400

ATP or intracellular inorganic phosphate, since the ratio of the P^{32} contents is the same at 48 hours as at 24 hours.

With respect to the inorganic phosphate, the principal consideration is the mechanism by which phosphate enters the cell from the extracellular phase. It is generally recognized that the cell membrane of muscle is impermeable to anions, yet in studies of diffusion of phosphate by the isotope technique, such as those of Hevesy and Rebbe (4) and Manery and Bale (6), the assumption is made, explicitly or implicitly, that phosphate enters the cell by simple diffusion, and is then converted to the organic compounds. The present data make such a position untenable, and show that phosphate enters the cell of striated or cardiac muscle only by being converted to an organic compound, presumably at the membrane. Conversely, phosphate can leave the cell only by the hydrolysis of these organic compounds at the membrane. This will be made clear by a

comparison of the P³² contents of plasma and *intracellular* inorganic phosphate with those of the PC and ATP.

<center>TABLE 3</center>

Distribution of radioactive phosphorus (P³²) in cardiac muscle of cats

Values are in terms of net counts per minute per milligram of P, calculated to the basis of 1×10^6 counts per minute injected, per kilogram body weight.

PLASMA INORG. P	HEART INORG. P	CALC. INTRA-CELLULAR INORG. P	PHOSPHO CREATINE	ADENO-SINETRI-PHOS-PHATE	HEXOSE MONO-PHOS-PHATE	ADENYLIC ACID	PLASMA INORG. P	HEART INORG. P
A. 1 hour after injection
							mgm. per cent	*mgm. per cent*
17,100	3260	*	1730	1780	725	154	6.5	10
14,050	1225	*	645	720	315	64	5.6	13
15,500	1690	*	570	460	200	29	6.3	10
7,800	900	*	286	228			7.9	10
Av.....13,612	1769		808	797	413	82		
B. 2 hours after injection
15,300	3100	392	1360	1200	390	640	6.9	12.5
18,800	3040	*	1985	2000	460	415	7.6	12
15,400	3010	82	2640	1870	1020	101	5.2	9
15,100	5680	1570	3160	3230	1090	110	5.5	6
Av.....16,150	3708		2286	2075	740	317		
C. 4 hours after injection
5,300	1920	865	1515	1465	1105	88	7.2	10
7,150	2000	730	1550	1310	1210	103	6.0	10
15,000	4270	2845	3090	2860	690	650	5.0	14
11,250	3210	2530	2430	2390	760	455	4.9	21
Av..... 9,675	2850	1743	2146	2006	941	324		
D. 24 hours after injection
547	1045	1150	980	990	650	600	5.2	10
915	1210	2100		1755	830	810	6.0	7
1055	1910	1930	1800	1670	2530	510	5.6	14
1115	1740	1840	1750	1880	1140	324	5.6	12
690	1340	1535	1145	1190	1050	475	5.6	8
735	1260	1390	1250	1380	1015	367	5.6	9
Av..... 843	1417	1654	1385	1478	1203	534		

* P³² content of extracellular fluid P less than that of plasma P.

The diffusion theory requires that the P³² content of the intracellular inorganic phosphate be higher than that of the PC or ATP while the plasma P³² is higher than that of the PC or ATP. It is obviously not possible to separate the intracellular inorganic phosphate from that in the extracellular phase, but the amount

present and its content of P^{32} can be calculated from the P contents of tissue and plasma, the P^{32} contents of plasma and tissue inorganic phosphate, and the volume of the extracellular phase. This last item has been determined by Amberson et al. (1) and by Yannet and Darrow (10), from the chloride contents of tissue and plasma. The two sets of data are in excellent agreement in assigning

TABLE 4

Distribution of radioactive phosphorus (P^{32}) *in resting muscles of frogs*

Values are in terms of net counts per minute per milligram P, calculated to the basis of 1×10^3 counts injected per gram body weight.

INORGANIC PHOSPHATE	PHOSPHO-CREATINE	ADENOSINE TRIPHOSPHATE	HEXOSEMONO-PHOSPHATE	ADENYLIC ACID	TOTAL P INJECTED GAMMA PER GRAM
A. 1 hour after injection					
254	72	72	123		21
437	65	91	150		21
218	54	49	98		21
Av......303	64	71	124		
B. 2 hours after injection					
156	26	35	195	15	3.4
118	67	57	114	14	2.7
187	92	73	139	18	17.0
374	280	195	183	28	16.7
410	78		135	11	26.0
248	59	54	168		15.4
Av......249	100	83	156	17	
C. 24 hours after injection					
110	142	128	53	41	3.3
115	125	115	33	11	3.8
Av......113	134	122	43	26	
D. 48 hours after injection					
98	105	103	51	18	17.0
101	110	112	32	17	19.2
102	115	97	40	17	18.5
288	298	304	68	51	12.3
Av......147	157	154	48	26	

an extracellular phase of 33 per cent to cat heart and 11 per cent to cat striated muscle. The values for the P^{32} content of the intracellular inorganic phosphate of striated and cardiac muscles of the cats shown in tables 1 and 3 have been calculated in this manner from the data there presented.

Inspection of these data shows that no such relation exists between the P^{32}

contents of the intracellular inorganic phosphate and the PC or ATP as would be required by the diffusion theory. The data on the cardiac muscles are particularly striking: in all but the 24-hour animals the intracellular inorganic phosphate has a much lower P^{32} content than do the PC and ATP. At 24 hours, the situation is reversed: the intracellular inorganic phosphate has the highest P^{32} content, while the PC and ATP exceed the plasma phosphate in P^{32} content. The observed relations are opposite to those required by the diffusion theory, and are in full accord with the hypothesis presented above, that phosphate enters or leaves the cell only by formation or breakdown of an organic compound at the membrane. The data on the striated muscles of the cats, in table 1, also lead to this conclusion, as do those on the frog muscles. In the 24- and 48-hour frogs the P^{32} of the inorganic phosphate is slightly below that of the PC and ATP. This condition could exist only if the plasma P^{32} fell below that of the intracellular inorganic phosphate.

The relations between the various phosphorus compounds of muscle and plasma phosphate can be represented by the following diagram:

```
Plasma                    Inorganic phosphate
                                  ↑↓
─────────────────────────────────────────────────────────
Capillary membrane                ││
─────────────────────────────────────────────────────────
Extracellular phase       Inorganic phosphate
                                              ↖
                          │        │        │ ╲╲
                          │        │        │   ╲╲
                          │        │        │     Adsorbed HMP
─────────────────────────────────────────────────────────
Cell membrane             ↓        ↓        ↓
                          ↑        ↑        ↑
                          │        │        │
Intracellular phase       PC      ATP      HMP
                          ↕        ↕
                        Inorganic Phosphate
```
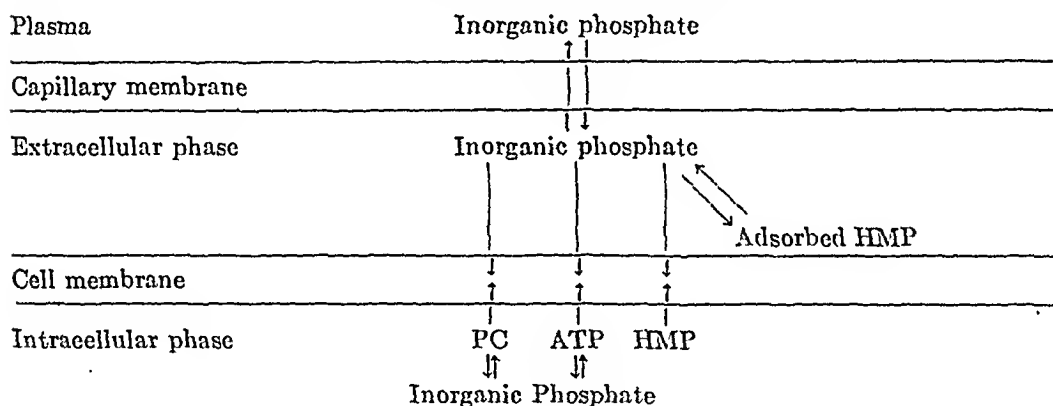
Additional evidence in favor of the view that phosphate enters the cell only by conversion to an organic compound at the membrane is found in the observation of Furchgott and Shorr (3) that in heart slices equilibrated with inorganic phosphate containing P^{32}, the intracellular inorganic phosphate and the PC have the same P^{32} content, at a level only one-fifth that of the medium. If the phosphate entered by diffusion, then at equilibrium the P^{32} content would be the same in the intracellular and extracellular phases.

With regard to the diffusion of phosphate through the capillary wall, the calculated values for the P^{32} content of the extracellular inorganic phosphate show that equilibrium is not established in one hour. On the assumption that all the P^{32} of the inorganic phosphate of cardiac or striated muscle is in the extracellular fluid, the P^{32} of this is between one-half and three-fourths as high as in the plasma P, at one hour after injection. Thus diffusion of phosphate through the capillary wall is appreciably slower than diffusion of sodium (6).

Granting that phosphate enters the cell only by conversion to an organic compound at the membrane, it becomes evident that PC and ATP exchange independently of each other at the membrane as well as with intracellular inorganic

phosphate.   If this were not the case, i.e., if either one were the sole source of exchange at the membrane and the other exchanged only with intracellular inorganic phosphate, then the P^{32} content of the second one would be dependent on the P^{32} content of the intracellular inorganic phosphate.   The data on the 4- and 24-hour animals show that this is not the case.   It therefore appears to be a purely fortuitous circumstance that the turnover rates of PC and ATP are so close together.

The HMP does not undergo any interchange with intracellular inorganic phosphate or with PC or ATP.   If such exchanges took place, then the P^{32} contents of the four intracellular compounds should become equal in time.   The data show clearly that this is not the case.

Reverting now to the finding of Furchgott and Shorr (3) that the P^{32} content of the intracellular inorganic P and PC of heart slices at equilibrium is one-fifth that of the phosphate of the medium, the most logical explanation would seem to be that, for each molecule of creatine which becomes phosphorylated at the membrane, 4 react with intracellular inorganic phosphate.   This ratio can, of course, be determined accurately only when the P^{32} content of the medium remains constant, as in experiments with slices, or by perfusion techniques. However, the present data on the intact heart indicate a ratio reasonably close to this.

Similarly, the 50 per cent turnover time can also be determined accurately only with a constant P^{32} level of the medium, but an approximation can be obtained in experiments on the intact animal from the time required for the P^{32} content of the substance in question to reach the maximum level.   The data on the heart indicate a 50 per cent turnover time of about an hour for the PC and ATP, and appreciably longer times for the AA and HMP.

In the case of the striated muscle, it is possible from the present data to make only the roughest sort of approximation of the 50 per cent turnover time or of the ratio of molecules of creatine or AA phosphorylated at the membrane and with intracellular inorganic phosphate.   The 50 per cent turnover time seems to be of the order of 4 hours; the ratio of phosphorylations at the membrane to intracellular phosphorylations is perhaps 1 to 50 or 1 to 100.

The turnover time of the HMP is extremely difficult to approximate.   Comparisons of the P^{32} contents of striated muscle HMP and plasma P at 4 hours after the injection indicates that the 50 per cent turnover time of the HMP is of the order of weeks, rather than hours.   In cardiac muscle the turnover is much more rapid.

Such rapid rates of breakdown and resynthesis of compounds in the resting metabolism of living tissue seem a good deal less startling at this date than they would have before the brilliant work of Schoenheimer on the rate of incorporation of heavy nitrogen into the tissue proteins.

The finding of such rapid interchanges of PC and ATP with inorganic phosphate in the resting metabolism of muscle offers some hope of reconciling the divergent views on the function of the phosphate compounds in contraction. In the formulation based on extract studies, the numerous phosphate interchanges have been directly connected with the formation of lactic acid, and it

has therefore been postulated that they were concerned with the formation of lactic acid in anaerobic contraction. The studies on the actual chemical changes in the contracting muscle, on the other hand, have found no evidence for the participation of the phosphate compounds in the formation of lactic acid. The present data indicate that if the phosphorylating glycolysis plays any part in the metabolism of muscle, it should be related to the resting metabolism, and not to the activity metabolism. This implies, of course, that the resting and activity metabolisms follow two radically different pathways. This is by no means the first indication of such a qualitative separation of the two types of metabolism. For contraction in the presence of oxygen, there is the finding of Stannard (9) that azide abolishes the excess oxygen consumption of the activity in concentrations which do not affect the resting oxygen consumption. For anaerobic contraction, there is the evidence (7) that the mechanism by which iodoacetic acid inhibits the formation of lactic acid in contraction is different from the one by which it inhibits the formation of this substance in extracts. This last point again brings attention to the possibility of a non-phosphorylating glycolysis in contraction, by way of methyl glyoxal. The iodacetate inhibition in this case might well be due to the destruction of reduced glutathione, the coenzyme of glyoxalase (5).

Granting that some of the reactions of the phosphorylating glycolysis may take place in the resting metabolism of muscle, it is evident that the entire cycle cannot be accepted without reservation. Participation of glucose-6-phosphate is ruled out by the finding that HMP does not interchange with PC, ATP or intracellular inorganic phosphate. However, there is no reason to rule out the possible formation of glucose-1-phosphate. If this were to undergo further reaction without being converted to the 6-phosphate, there would be no barrier to the acceptance of the phosphorylation cycle as the mechanism of aerobic glycolysis in the resting metabolism of muscle. There would then be no occasion to postulate that it is operating in contraction, irrespective of whether this takes place in the presence or absence of oxygen. Such a situation would still leave unsolved many problems in both fields. The function of phosphocreatine in the resting metabolism is apparently more important than the most recent developments of the phosphorylation cycle would indicate; furthermore, the mechanism of lactic acid formation in anaerobic contraction still remains to be elucidated.

SUMMARY AND CONCLUSIONS

1. The time course of the distribution of radioactive phosphorus in resting striated muscle has been studied in cats and frogs, and in the heart in cats.

2. The diffusion of phosphate through the capillary wall is relatively slow in comparison to other ions which have been studied. Equilibrium between plasma and extracellular fluid is not reached in one hour.

3. Phosphate enters the interior of the cell of striated or cardiac muscle only by being converted to an organic compound at the membrane, and leaves the cell only by the dephosphorylation of these compounds at the membrane.

4. The formation of phosphocreatine, adenosine triphosphate and hexose-monophosphate are independent processes, rather than being part of a cycle.

5. The phosphorylation of creatine and adenylic acid may take place either at the cell membrane or with inorganic phosphate present within the cell.

6. For each molecule of creatine or adenylic acid which is phosphorylated at the membrane, there are approximately 4 molecules phosphorylated intracellularly, in the heart, and a much larger number in striated muscle.

7. The hexosemonophosphate of striated or cardiac muscle does not interchange, directly or indirectly, with intracellular inorganic phosphate, phosphocreatine or adenosine triphosphate.

8. The turnover rate of phosphocreatine and adenosine triphosphate is independent of the amount of phosphate injected.

9. When large amounts of phosphate are injected there is a temporary accumulation of hexosemonophosphate on the cell membrane of striated muscle.

10. There is a rapid breakdown and resynthesis of phosphocreatine and adenosine triphosphate in the *resting* metabolism of striated and cardiac muscle.

11. There is evidence that some, but not all, of the reactions of the phosphorylating glycolysis that have been found in cell-free muscle extracts may take place in the *resting* metabolism, but not in the *activity* metabolism, of striated and cardiac muscle.

12. The chemical transformations associated with the contraction of striated and cardiac muscle are qualitatively different from those concerned with the resting metabolism.

REFERENCES

(1) AMBERSON, W. R., T. P. NASH, A. G. MULDER AND D. BINNS. This Journal **122**: 224, 1938.
(2) BARKER, S. B., R. F. FURCHGOTT AND E. SHORR. Ibid. **133**: P.202, 1941.
(3) FURCHGOTT, R. F. AND E. SHORR. Fed. Proc. **1**: 27, 1942.
(4) HEVESY, G. AND O. REBBE. Acta Physiol. Scand. **1**: 171, 1940.
(5) LOHMANN, K. Biochem. Ztschr. **262**: 152, 1933.
(6) MANERY, J. F. AND W. F. BALE. This Journal **132**: 215, 1941.
(7) SACKS, J. Ibid. **126**: 388, 1939.
(8) SACKS, J. Ibid. **129**: 227, 1940.
(9) STANNARD, J. N. Ibid. **126**: 196, 1939.
(10) YANNET, H. AND D. C. DARROW. J. Biol. Chem. **134**: 721, 1940.

EFFECTS OF VISIBLE RADIATIONS UPON ALBINO RATS

JOHN ALLARDYCE, JOHN ALDOUS, WILLIAM COOPER, JEAN PRATT AND
ERNEST SUTHERLAND

*From the Department of Biology and Botany of The University of British Columbia,
Vancouver, B. C.*

Seasonal fluctuations in the weight of animal organs have been noted by a number of investigators. Brown, Pearce and Van Allen (2) maintained rabbits in environments of constant illumination, solar illumination and darkness and noted that those under constant illumination tended to have organs of relatively smaller weight than those of the controls and that the periodic fluctuations in the size of the organs were absent. Over a period of four years, Del Castillo and Pinto (3) observed a periodic fluctuation in the size of the testes and the prostates of rats. Following extensive work on the effect of light upon the sexual development of birds, Bissonnette (1) concluded that the progressive changes in the testes in the spring may be helped by the relative strength of red rays in the sunlight of that season and that the natural regression in summer and early autumn may be due to the relative excess of inhibiting or lethal green (and perhaps violet and ultraviolet rays) of this season. In 1939, Luce-Clausen and Brown (5) found that visible radiations promoted growth of rats, that the opening of the vagina and onset of oestrus were delayed in rats confined to darkness, and that the survival of young rats was higher under visible radiations. In 1941 Fiske (4) reported that female rats, kept under light from birth or from the twenty-first day of life, matured much earlier than females kept in darkness, that unusually long oestrus periods were characteristic of the former while frequent metoestrus occurred among the latter, and that the pituitaries, ovaries and uteri of 77 day old rats, kept under light for 8 weeks, were heavier than those of litter mates kept in darkness.

In the present investigation an attempt has been made to ascertain whether some portions of the visible spectrum are more effective than others upon growth, activity, basal metabolism, reproduction and survival of litters of albino rats.

METHODS. Cages were set up on shelves 7 feet away from and facing two 100 watt Mazda electric bulbs. The front and top of these cages consisted of $\frac{3}{8}$ inch mesh galvanized wire while the sides and back were made of galvanized sheeting. In front of each cage was placed a cellophane filter, either black, colorless, red, orange, yellow, green, blue or violet. With the exception of the black, these filters consisted of a single thickness and are described by the manufacturers, Canadian Industries Limited, as no. 300 plain transparent, red, tango, amber, light blue and violet respectively. The percentage of light transmitted and the region of transmission for each of the colored filters was determined[1] and is shown in figure 1. The black filters consisted of a heavy sheet of

---

[1] This determination was made possible through the co-operation of the Department of Physics and was carried out by Thomas Collins.

kraft paper covered on both sides with black cellophane. To eliminate any seasonal influence, the temperature of the rat room was thermostatically controlled at 70°F. and the period of illumination electrically controlled at 10 hours per day.

Three series of Wistar albino rats have been used in this work. In the first series, an exploratory run begun in 1939, 8 males and 8 females at 5 weeks of age were placed in pairs behind the 8 filters described above and carried for 190 days. In the second series, begun in 1940, 27 rats at 3 weeks of age were avail-
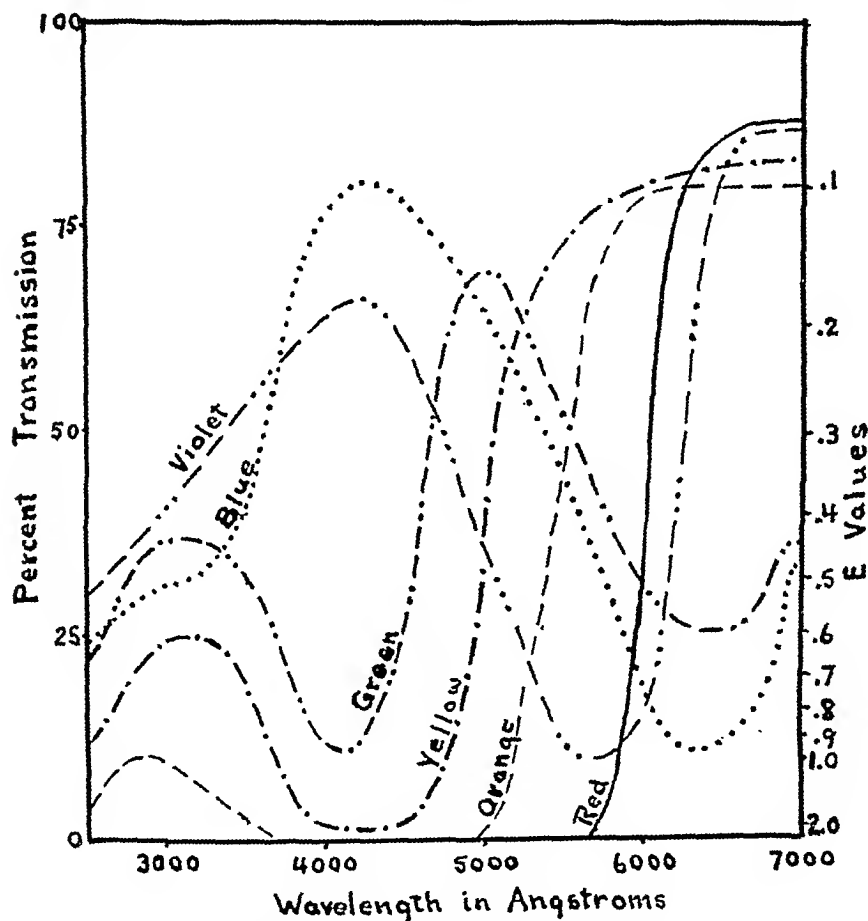
Fig. 1. Light transmitted by cellophane filters.

able. Behind each of the 8 filters were placed 1 male and 2 females. This left 3 more females which were placed behind the red, blue and violet filters. A few weeks later an error in sexing the rats was discovered. Behind the colorless filter there were 2 males and 1 female. This second series was carried for 212 days. The third series, begun in 1941, consisted of the F_1 generation of the second series with the exception of those behind the blue filter. Since no litters had been born behind the blue filter, rats for this filter in the third series had to be drawn from new stock. This series was started when the rats were 3 weeks old. Behind each filter were placed 1 male and 2 females. Two cases of incorrect sex-

ing were later discovered in this series, behind the colorless and the red filters where in each there were 2 males and 1 female. After carrying this third series for 180 days, the filters were changed so that rats, previously behind colorless or colored filters were placed behind black filters while those behind black filters were placed behind blue filters. Following this change the series was carried another 210 days; at the end of this time the original filters were restored and the series completed by the end of 21 days more.

Each adult rat was given three Purina Fox Chow checkers per day. While nursing its litter each female was given two additional checkers per day. Occasionally all rats received supplements of carrots and green leafy vegetables.

At the time of casting of a litter the other rats in that cage were removed to another behind a similar filter. When the litter was 3 weeks old, the mother was returned to her original group.

Growth was determined from weight increases. All rats were weighed once a week between the ages of 21 and 70 days. Thereafter the males were weighed once a week while the females were weighed twice a week. Each litter was handled as little as possible and consequently was weighed as a whole at birth and at 3 weeks of age.

Activity measurements were attempted with two pieces of apparatus constructed for the purpose. In one of these, initial activity (the first 90 sec.) was sought, while in the other activity over a 24 hour period was investigated. In both cases the tests were carried out 18 to 24 hours after feeding.

The apparatus used for determining initial activity consisted of a rubber diaphragm stretched tightly over a four-inch funnel by means of twelve small battery clamps anchored to an iron retort ring at a lower level. Fitting around the edge of the funnel and extending upward 14 inches a cardboard cone served to retain the rat on the rubber diaphragm. The stem of the funnel was connected by rubber tubing to a tambour. The thin rubber membrane on the tambour was fastened securely but not stretched, in order to permit greater movement of the writing lever attached which recorded on a smoked drum revolving once in 90 seconds. Because of the friction of the writing lever on the drum and the elasticity of the air in the apparatus, it was found that greater movements were recorded on the drum when the air in the apparatus was replaced by water. Each rat was dropped head first from just above the cardboard cone and the movements recorded. The preserved records were enlarged by projection on a translucent screen and the length of the activity line minus the base line determined.

The second activity apparatus consisted of a chamber 24 in. wide, 8 in. deep and 30 in. high. It was divided into an upper, middle and lower chamber by three boards constituting the floors of these chambers. These boards were cut across the middle and hinged there. The outer ends of each of these were slightly raised by a light spring. As a rat moved out towards the end of one of these boards, the outer end descended 1 cm. and in so doing passed an electrical contact. This caused a signal magnet to record the movement and also caused an electro-

magnet to rotate the smoked double drum about 2 mm. When the rat moved back towards the center of that floor another contact was made as the outer end of the board rose and this movement was recorded. In like manner movements of the other boards at each level moved signal magnets and were recorded. A sloping runway permitted the rat to move from one level to the next. A seventh signal magnet attached to a clock recorded one hour intervals.

Basal metabolism was determined in an apparatus constructed, with a few modifications, on the plan of Schwabe and Griffith (6). The chief modification was the manner of drawing oxygen through the apparatus and of removing carbon dioxide. By lowering and raising a reservoir Schwabe and Griffith caused the air in the rat chamber to be brought in contact with a standardized barium hydroxide solution for removal of the carbon dioxide and the return of the residual air to the rat chamber. In the present investigation the rat chamber consisted of a flat-bottomed cylindrical glass vessel 9 in. in diameter and 3 in. deep. This was inverted in a narrow circular mercury trough on a varnished copper plate. Coming through the floor of the chamber was a sealed-in copper tube connecting the chamber with the rest of the apparatus and delivering oxygen to the chamber at a rate equal to the rate at which it was consumed by the rat. The carbon dioxide and water vapor set free by the rat were absorbed by soda-lime placed between two pieces of copper gauze to form a wall $2\frac{1}{2}$ in. high and $\frac{3}{4}$ in. thick extending around the chamber just inside the glass wall. The wire gauze was held in place by small wooden posts $2\frac{1}{2}$ in. long nailed in an upright position to a thin circular wooden disc which fitted inside the chamber. In recording the rate at which oxygen was utilized by the rat, a cylindrical glass float was substituted for the paraffined cork used by Schwabe and Griffith. This eliminated small fluctuations in the oxygen curve which appeared with the formation and collapse of each oxygen bubble due to the oversensitiveness of the cork float. The glass float substituted, while not registering these, was quite sensitive to any change in activity of the rat, as seen by an increase in the slope of the oxygen curve with the slightest movements of the rat. Construction and testing of this apparatus was completed in April 1942. This has permitted its utilization in carrying out basal metabolism determinations in the third series of rats under two sets of conditions. In the first of these, the rats, after 180 days behind the 8 types of filters, had been switched to the black or the blue filters, as previously described, and were in the last month of this 210 day period. The second set of conditions covers the final 21 day period of this series during which the rats were behind their original filters.

RESULTS AND DISCUSSION. *A. Growth.* After studying the growth curves of the rats in the three series, it became evident that there was no consistent difference, either in the weights of the rats at any particular age or in the rates of growth, that could be attributed to the filters used.

B. Activity. Neither activity apparatus was completed in time to be used with the first series of rats. In the second series, initial activity graphs were obtained but no satisfactory method developed for determining quantitatively the relative activities expressed by these graphs. However, inspection of these

graphs definitely placed the rats behind the red and the black filters as the most active, those behind the orange the least active, and the others in an intermediate position. In the third series, the relative initial activities were determined quantitatively and are summarized in table 1. With the exception of the position of the rats behind the yellow filter, which have moved from an intermediate to the top position, the order of activity remains practically the same as the second series.

Rats placed in the second activity apparatus for the 24-hour test showed activity only in the first 4 hours and the greater part of this in the first 2 hours.

TABLE 1

Initial activity

	FILTER							
	Yellow	Red	Black	Green	Colorless	Blue	Violet	Orange
Activity..........	102.3	72.4	66.8	57.3	43.5	40.0	37.2	31.5

TABLE 2

Basal metabolic rates

	FILTERS							
210-day period Original color.	Yellow	Red	Black	Violet	Orange	Blue	Colorless	Green
to............	Black	Black	Blue	Black	Black	Black	Black	Black
B. M. R. (a)..	52.9	44.8	43.8	43.4	43.0	41.2	40.1	40.0
21-day period Restored to...	Yellow	Blue	Black	Violet	Red	Orange	Colorless	Green
from..........	Black	Black	Blue	Black	Black	Black	Black	Black
B. M. R. (b)..	41.4	39.5	38.7	37.1	36.4	35.8	32.2	30.6
Original color...	Yellow	Green	Red	Colorless	Orange	Violet	Black	Blue
Drop in B. M. R........ (a − b)	11.5	9.4	8.4	7.9	7.2	6.1	5.1	1.7

These tests, therefore, were reduced to 4 hours. However, the variation in response of the same rat on repeated tests on different days was too great to make use of such tests for measuring activity.

C. Basal metabolism. The basal metabolic rate for each rat was determined on three separate occasions during the last month of the 210 day period. From these the average for each rat was determined and in turn an average worked out for all rats getting the same radiation. It is this last group of figures which is shown in table 2. In like manner for the 21 day period after returning the rats to their original filters, three determinations were made with each rat and an average taken. Then from these individual averages, averages for all

rats getting the same radiation were determined. In calculating the surface area of the rat, Rubner's formula was used. The basal metabolic rates are expressed as kilogram-calories per square meter per hour.

It will be recalled from table 1 that rats behind the yellow filter were the most active. From table 2, this same group has the highest basal metabolic rate. Further, all groups showed a lower B. M. R. when returned to their original filters for 3 weeks from black filters, or from behind blue in the case of original blacks. This drop in B. M. R. was greatest for the yellow filter followed closely by green, red and colorless and was least in restoration of blue from black.

D. Reproduction. In the first series of rats litters were cast behind all filters except the blue. Autopsies at the end of the test period, 190 days, revealed no developing feti in the rats behind the blue filter. Toward the litters receiving radiations through the different filters there appeared to be, in general, less care given by the mother and a greater cannibalistic tendency than usual and this occurred independently of the size of the litters.

In the second series of rats these points were investigated further. At the completion of this series, 212 days, all females, with the exception of those behind the blue filter, had cast litters. At this stage, two of the three females behind the blue filters were given three intramuscular injections of 1 cc. each, at 3-day intervals, of oestradiol benzoate in sesame oil (0.2 mgm per cc.).[2] At the same time the male was given like injections of testosterone propionate in sesame oil (5 mgm per cc.).[2] By the end of a further 90 days there still were no litters cast behind the blue filter.

Since this was the second group of rats which had been raised behind the blue filter and had failed to cast a litter, it seemed unlikely that chance sterility was the cause. However, to be sure of this a third group was started behind the blue filter and placed in the third series. At an age of 180 days this third group of rats behind the blue filter had cast no litters. At this stage, switching the blue filter for a black over an additional 210-day period, did not alter the situation as far as litters were concerned.

In both the second and third series of rats it will be seen by reference to table. 3 that rats raised behind the black filter stood well up in the list with regard to the number of offspring per rat but were bettered by rats behind the yellow filter. On the other hand, reproduction behind the colorless filter appeared to have been repressed.

In the last column of table 3 the colors given refer to the original filters. It will be recalled that these were switched so that rats behind black were given blue and all other filters were replaced by black. Further, with the exception of rats behind the original blue filter, all in the third series belong to the F_1 generation of the rats in the second series. It should also be noted that the length of the first part of the third series is a month shorter than the balance of that series or the full second series. Keeping these points in mind, it would appear that rats behind the yellow filter in both series had the same degree of fertility and this

---

[2] We are indebted to Ciba Co. of Montreal for these preparations.

was not affected by the switch of filters from yellow to black.   Rats behind the black filter showed approximately the same fertility in the second series and first part of the third but with the switch to blue in the second part of the third series their fertility dropped about 25 per cent.   Rats behind the violet filter in both series had about the same fertility until the filter was switched to black when the fertility dropped more than 50 per cent.   A similar story holds for green with an even greater drop in fertility after switching to black.   Rats behind the red filter showed a sharp decrease in fertility in the F_1 generation

TABLE 3

Number of offspring per rat

SECOND SERIES		THIRD SERIES			
212 days		First 180 days		Next 210 days (filters switched)	
Yellow	24.0	Yellow	17.0	Yellow	23.5
Red	23.7	Black	16.5	Black	18.5
Violet	22.0	Violet	13.0	Violet	9.5
Orange	22.0	Green	8.0	Green	3.0
Black	20.5	Red	3.0	Red	0
Green	13.5	Colorless	2.0	Colorless	0
Colorless	4.0	Orange	0	Orange	0
Blue	0	Blue	0	Blue	0

TABLE 4

Percentage survival to 21 days in litters

SECOND SERIES			THIRD SERIES			
212 days			First 180 days		Next 210 days	(Filters switched)
Black	5/41	83.0%	Yellow	5/34	88.2%	Green	1/3	100.0%
Colorless	1/4	75.0	Black	4/33	81.8	Yellow	7/47	97.9
Violet	9/66	74.3	Violet	3/26	80.8	Violet	4/19	89.5
Green	5/27	63.0	Green	3/16	68.8	Black	5/39	45.9
Red	7/71	55.6	Colorless	2/4	50.0			
Orange	5/44	40.9	Red	1/3	0			
Yellow	6/48	39.6						

with no litters cast in the last 210 days.   While rats behind the orange filter in the parent generation produced well, no litters were cast in the F_1 generation.

This decreasing fertility exhibited in the third series and particularly in the 210-day period can not be due entirely to the increasing age of the rats for at the end of the 210-day period they were only 390 days old.   Further, if age were the controlling factor, the effects would be more evenly distributed over all the test animals.   It would seem that radiation is a factor, possibly the prime one in this case, since behind black filters fertility of the rats was maintained while behind all other filters used, except yellow, fertility decreased at different rates and to

different degrees.   After 180 days' radiation, this decreasing fertility was not halted by placing the rats behind black filters.

E. Survival of litters.   The effect of using the different filters on the survival of the litters to 21 days is shown in table 4.

As in table 3, the colors in the third column refer to the original filters used. Where no litters were born, the filters are not mentioned.   The number of litters cast and the total number of offspring in these litters is respectively indicated by the numerators and denominators of the fractions placed immediately after the color of each filter.   In four cases the survival values are based on a rather limited number of offspring and should be considered with this in mind.   The cases referred to are the colorless in the second series, the colorless and the red in the first part of the third series, and the green in the last part of the third series.

Consideration of the survival values shown for the second series and first part of the third places the black filter at the top with a value of 82.4 per cent, followed by violet (77.5), green (65.9), yellow (63.9), colorless (62.5), orange (40.9) and red (27.8).   Whether this order is coincidental or significant, it is nteresting to note that it is the same as in the solar spectrum.   The second part of the third series would seem to further establish the position of the black filter toward favoring the survival of litters, since switching green, yellow and violet to black improved survival values while switching black to blue considerably owered the chance of survival.

SUMMARY

Growth, initial activity, basal metabolism and reproduction of albino rats and the survival of their litters behind black, colorless, red, orange, yellow, green, blue and violet filters have been compared.

No consistent difference either in the weights of rats at any particular age or in their rates of growth was found which could be attributed to the color of the filter used.

Initial activity was influenced by the color of the filter used, being greatest under yellow, followed by red and black and least under orange.

Basal metabolic rates were highest under the yellow filter and lowest under the green.

The number of offspring per rat was highest under the yellow filter throughout the experiment while under the blue filter reproduction was inhibited from the start.   The other filters exhibited degrees of inhibition.

Survival of litters to 21 days was highest behind the black filter and lowest behind the red.

REFERENCES

(1) BISSONNETTE, T. H.   Physiol. Zool. **5**: 92, 1932.
(2) BROWN, W. H., L. PEARCE AND C. M. VAN ALLEN.   J. Exper. Med. **42**: 69, 1925.
(3) DEL CASTILLO, E. B. AND A. PINTO.   Rec. Soc. Argentina Biol. **5**: 463, 1939.
(4) FISKE, V. M.   Endocrinology **29**: 187, 1941.
(5) LUCE-CLAUSEN, E. M. AND E. F. BROWN.   J. Nutrition **18**: 537, 1939.
(6) SCHWABE, E. L. AND F. R. GRIFFITH.   J. Nutrition **15**: 187, 1938.

THE RELATIONSHIP BETWEEN MONOCHROMATIC LIGHT AND PUPIL DIAMETER. THE LOW INTENSITY VISIBILITY CURVE AS MEASURED BY PUPILLARY MEASUREMENTS

I. H. WAGMAN AND J. E. GULLBERG

From the Division of Physiology, Medical School, and the Department of Zoology, University of California, Berkeley

It has been known for some time that there is a considerable degree of correspondence between pupillomotor and sensory effects of stimulation of the retina by light. Reeves in 1918 showed that during dark adaptation, after previous exposure of the eye to a bright light, the curve for pupillary dilatation closely parallels that for increasing retinal sensitivity. Others subsequently found this to be true (Crawford, 1936; Brown and Page, 1939, both on humans; Gullberg, Olmsted and Wagman, 1938, on rabbits).

This correspondence is further seen in studying the effect of different wave lengths of light on the size of the pupil. Although these studies have been few, they do point to the fact that the visibility curves of the eye obtained by measuring the variation in pupil diameter in the different wave lengths are comparable to those obtained by the more usual subjective means. Laurens (1923), as Sachs (1892) and Abelsdorff (1900) had done earlier, determined the visibility curves of the human eye using the pupil diameter as a measure. Hess (1913, 1915) studied the effects of different spectral lights and of the lights transmitted by colored glasses on the pupil reactions of cats and rabbits. He found that the maximum pupil constriction for these animals in the light adapted state was at about 550 mμ and in the dark adapted state was at about 505 mμ. Laurens (1923) determined the visibility curves of both the pigeon and the alligator by means of pupillary measurements. In the pigeon the maximum effect in the light adapted eye was at 564.1 mμ, and in the dark adapted eye at 544.2 mμ. In the alligator eye these maxima were at 544.2 mμ and 514.2 mμ respectively.

Hecht and Pirenne (1940) investigated the retinal sensitivity of the nocturnal long-eared owl, using the pupillary size as a measure of sensitivity. They measured the relative effectiveness of different wave lengths of light in causing a constant amount of pupil contraction, and found that the spectral visibility curve for the owl is the same as the human visibility curve at low light intensities, with a maximum at about 515 mμ.

These studies lead to the conclusion that pupillomotor action depends upon two receptors, just as does retinal sensitivity; namely, the rods and cones. This view is supported by the results of other studies, the majority of which point to the fact that both receptors play a part in the regulation of the size of the pupil, although the effect of the cones predominates. (cf. Laurens, 1923; Ferree, Rand and Harris, 1933, and Brown and Page, 1939, for reviews on this subject.)

Brown and Page (1939) concluded from a study of dilatation of the pupil in darkness that the pupil size relative to light stimulation is under the control of fibers activated only by cones in the central portion of the retina. These two workers show that the course of pupillary dilatation as measured by them, by Reeves (1918) and by Crawford (1936), bears a strong relationship to foveal dark adaptation as measured by Hecht (1921a) and by Hecht, Haig and Chase (1937). Pupil dilatation, just as foveal dark adaptation, is almost complete in about one minute, and practically all completed in five or six minutes, and fully complete in ten minutes at the most. Brown and Page believe that the Purkinje effect would result from transmission of excitation from the rods within or outside the macula to optic nerve fibers leading off from cones and controlling pupillary constriction, and therefore that the rods have no influence on the movements of the pupil.

An attempt is here made to measure the low intensity human visibility curve using the objective measure of pupillary size. If the rods have any effect on the pupillary response, we should be able in this manner to detect a rod influence if it exists.

The work which has been done on spectral sensibility of the human eye using the pupil as a measure has been slight and, in general, unsatisfactory. Although the work of Laurens (1923) remains the best of the studies done on this particular problem (cf. Sachs, 1892, and Abelsdorff, 1900) there is a very significant objection to it. Laurens determined the amount of pupillary contraction in the pigeon and alligator as well as in the human caused by different wave lengths of equal energy. According to the classical accepted definition of a spectral luminosity curve, this latter study is not accurate. Such a curve, to be acceptable and comparable to other investigations, must record the reciprocal of the relative energy required in different parts of the spectrum to produce the same physiological effect (Hecht, 1928; Graham and Hartline, 1935; Hecht and Pirenne, 1940).

METHOD. Infrared photography is the most suitable method for accurately measuring the diameter of the pupil in virtual darkness as well as under any condition of light adaptation. Infrared light does not influence the size of the pupil (Crawford, 1936; Gullberg, Olmsted and Wagman, 1938; Brown and Page, 1939). This method can also be satisfactorily used when a beam of colored or white light is directed into the eye.

The apparatus devised for these experiments consisted essentially of three distinct parts:

1. An instrument for adapting the eye to a spot of colored light of any intensity within the necessary range, and having any diameter up to about 16.6 degrees visual angle (fig. 1).

2. A camera and auxiliary apparatus for photographing the pupil (figs. 1 and 2).

3. An infrared source of light which is used to illuminate the eye when photographing.

The adapting instrument used fulfills all the specifications needed to pursue work on both light and dark adaptation (Hecht and Shlaer, 1938). The intensity, the color, and the duration of the preadapting light may be varied to suit any of

the conditions of the experiment.   Provisions are made to vary the area and retinal location of the light in the eye.   This instrument can also be used for subjectively measuring the visual threshold after any period of dark adaptation.

The instrument is shown diagrammatically in figure 1.   The source of light is the circular ground glass window, G, 20 mm. in diameter, illuminated by a 6 volts, 18 amperes, coiled filament projection bulb, T, which has been seasoned.
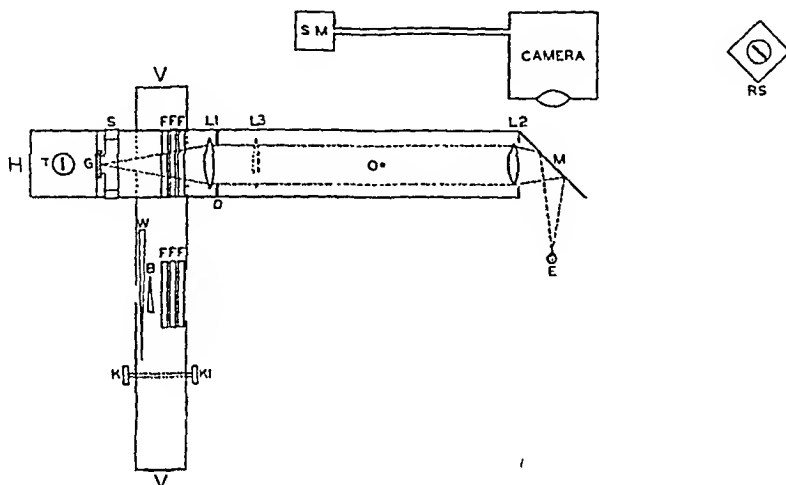
Fig. 1.   Explanation in text
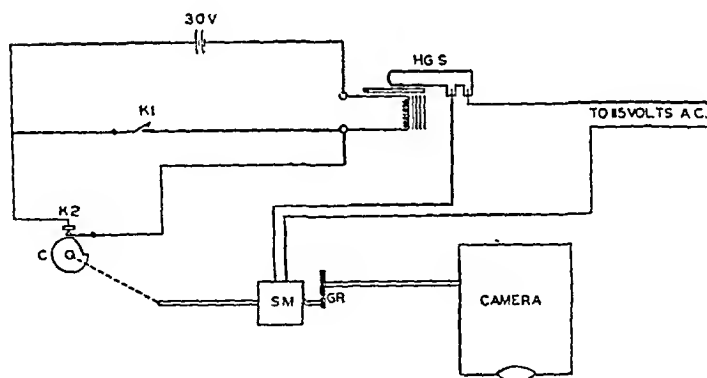
Fig. 2.   Explanation in text

The lamp burned at 6.2 volts and its operating characteristics were checked at frequent intervals.

The lens $L 1$, 60 mm. in diameter, is placed at its principal focal length of 12 cm. from the secondary source of ground glass G.   The beam of approximately parallel light is picked up by another lens, $L 2$, having a diameter of 48 mm. and a focal length of 15 cm.   It is then reflected through an angle of 90 degrees by the partially aluminum-sputtered mirror, M.   Thus an image of the source G is produced in the plane of the pupil of the eye at E, and the lens $L 2$ appears uniformly illuminated and seems to be placed directly in front of the observer.

Near the lens $L\,2$ is a groove, D, which is made to hold metal diaphragms of varying sizes in order to control the beam of light. The maximum visual angle obtainable is 16.6 degrees with a diaphragm of 44 mm. opening.

$L\,3$ is a lens of 60 mm. in diameter and 45 cm. focal length which can be substituted for $L\,2$. When it is in place without $L\,2$ and when used with a maximum size diaphragm at D, an image of the source is produced having a visual angle of 5.7 degrees.

A Compur shutter, S, is placed in front of the light source and is used to regulate the exposure time from $\frac{1}{50}$ of a second up to minutes of exposure.

V is a movable mount to carry colored and neutral filters, F, neutral wedge, W, and balancing wedge, B. This carrier can be displaced in such a manner as to permit utilization of full intensity of light or of the different possible combinations of neutral filters and wedge to give an intensity range of from 1 to 10^{-12}.

Six Wratten neutral filters were used transmitting from $\frac{1}{2}$ to $\frac{1}{10\,000}$ of the incident light. The wedge is 15 cm. long and covers an intensity range of from 1 to 1000. It is used in conjunction with a balancing wedge so as to provide a uniform field.

The neutral wedge is moved by a rack and pinion, and has a scaled dial, gear connected so as to define the position of the wedge. To vary the spectral characteristics of the light the special set of Wratten Monochromatic Filters was used. This set has been extensively used in objective studies where the high intensities required were not easily attained with a spectroscope (Hecht, 1921b; Crozier, 1924; Hecht, 1928; Grundfest, 1932a, b; Graham and Riggs, 1935; Graham and Hartline, 1935; Hecht and Pirenne, 1940).

The eye when in a position to be adapted and photographed was fixated on a target, O, which was placed 10.5 cm. in front of lens $L\,2$, giving a virtual image 45 cm. from the eye. The fixation point was a May Ophthalmoscope bulb blackened except for a small point which provided a red window. The brightness of this small red dot was controlled by a rheostat so as to keep its intensity at or slightly above the threshold of the subject.

Since 45 cm. is within the accommodation power of the emmetropic eye, the question arises as to whether the pupil diameter is influenced by near accommodation, and if so, to what extent. Many subjects used here were myopic with a refractive error of -2.25 D or greater, so that in fixating at 45 cm. they relaxed accommodation, and their pupil diameter was not influenced. Luckiesh and Moss (1934) found that for the emmetropic eye, at a brightness level of 0.1 milli-lambert, the pupil size remained at its maximum at fixational distances of 60 cm. and beyond. At 45 cm. the pupil reached a diameter which was only slightly less than the maximum.

In any event this was kept a constant factor in these studies. Attempts were made to learn whether an increased pupillary size could be obtained by relaxing accommodation in the dark adapted eye. The effect was found to be negligible.

The camera used to photograph the pupil of the eye was the model J, Debric 35 mm. motion picture camera. It was provided with a Zeiss Biotar 50 mm. focal length, F 1.4, mounted so as to permit photography at a distance of 15 cm.

The camera was driven by a rod mechanism geared to a synchronous motor, SM, in figure 2. The gear ratio was 1 to 10 (GR) and when the camera was set to operate at one film per revolution, the complete cycle for taking a photograph was 4.2 seconds. A hand key, $K1$, initiates the camera cycle by operating a mercury switch, $HG.S$. The rotation of the camera drive actuates a cam mechanism, C, which closes a parallel electrical circuit $K2$ to continue rotation until a position is reached where the photograph has been taken and the camera is arrested in preparation for the next cycle.

The exposure time is varied by the opening or closing of the angular aperture of the camera shutter. With Eastman infrared film it was found that a lightly sputtered mirror required an angular exposure setting of the order of $\frac{1}{8}$ of a second.

The infrared source of light shown as RS in figure 1 has been fully described elsewhere (Wagman, 1937: M.A. Thesis, Univ. of Calif. Library, Berkeley; Gullberg, Olmsted and Wagman, 1938). A 30-volt, 30 ampere motion picture projection lamp was used to obtain a concentrated beam of light emitting a large proportion of infrared energy. The tungsten lamp is used with a spherical reflector and two B. and L. Cinephor aspheric condensing lenses. A Wratten no. 87 filter was mounted 10.5 inches in front of the lenses, the whole unit being enclosed in a light-tight box provided with adequate baffles for good air circulation. The 900 watt lamp was controlled by a carbon plate rheostat and a Weston wattmeter. When adjusted to operate at 400 watts such a lamp and filter contain only a small component of visible red in addition to infrared. The eye was illuminated at an angle of about 45 degrees and it was found that this residual visible red had no measurable effect upon the pupil. The subject's head was held in position by a chin rest used in conjunction with a rest for the cheek. When the subject's eye was fixated above the target, the beam of light from the adapting instrument focused immediately upon the pupil which was also in focus for the camera. The eye could thus remain in a constant position during the entire length of the experiment. The adaptometer was well shielded by a wooden hood and since the infrared source is essentially light-tight in the visible range, the experiments were performed in complete darkness. The experimenter used a small flashlight provided with a red bulb to read instruments and check positions.

The time interval in the course of pupillary dilatation was checked either by observing a watch or using a signal magnet connected in series with key, $K1$, and a time trace on a kymograph drum.

At the beginning of each photographic series a millimeter scale was placed in the plane equivalent to the pupil position and photographed. This provided a reference scale for the measurement of pupil size. The films were developed in Eastman "D-19" developer to a considerable degree of contrast. These negatives used with a "Brinell" measuring microscope gave pupil diameter with an accuracy of a fraction of a millimeter.

Calibrations. The brightnesses of white light from the adapting instrument obtained with and without each of the neutral filters, and for fifteen points on the neutral wedge as well, were measured at the plane of the pupil where a piece

of white blotting paper was placed. The reflectance of this paper for white light had been accurately calibrated by the Department of Mechanical Engineering. The brightness on the blotting paper surface of each particular light intensity was measured by means of a Luckiesh and Taylor Brightness Meter placed three feet away from this surface. After accounting for the reflectance loss on the blotting paper surface, the apparent brightness at that point was obtained in foot-lamberts. From them the brightnesses given by any combination of filters and wedge could be determined.

The Wratten Monochromatic filters were calibrated by an indirect method which has been used successfully by others. In addition to its being quite accurate, the method eliminates the effect of the infrared radiations which each of these filters transmit (Hecht, 1928; Grundfest, 1932a, b; Graham and Hartline, 1935; Hecht and Pirenne, 1940).

The method consisted first in measuring the percentage transmission of each of the filters for every ten milli-microns between 400 and 700 milli-microns,[1] and the relative energy distribution in the spectrum of the 108 watt lamp while it was in its usual place in the adapting apparatus. The latter measurement was made by determining the color temperature of the lamp in conjunction with the ground glass, the lenses, and the partially aluminated mirrors as they are set in the instrument. The color temperature was found to be 2980 degrees Kelvin[2].

The relative energy of the lamp at any wave length multiplied by the transmission of a filter at that wave length gives the amount of energy transmitted by the filter at that particular wave length. For each filter the energy transmission which was obtained for every ten milli-microns in the visible part of the spectrum only was plotted against wave length. The total relative energy transmitted by each filter was determined by measuring the area under its transmission curve by means of a planimeter.

The wave length which divides the curve into two equal areas was then determined planimetrically. This wave length corresponds to the center of the energy transmitted by each filter in conjunction with the lamp in the system, and is the wave length used in dealing with the results obtained. The information gathered by the above methods is given in table 1.

The calibrations obtained by the indirect method described were checked roughly, by means of both a thermopile and Weston photronic cell, each used with a sensitive galvanometer. The results obtained by both these latter methods agreed well with those shown in table 1.

The procedure used in studying the effect of white light on pupil diameter described by Wagman and Nathanson (1942) was used here as well. The effect of each of the seven wave lengths was measured at nine different points covering a range of from about five log relative energy units (at 688 mμ) to about eight

[1] We thank Dr. G. Mackinney of the Division of Fruit Products in the College of Agriculture for allowing us to use the Bausch and Lomb spectrophotometer and for his help in determining these transmission values.

[2] These measurements were kindly made by Mr. A. Collins of the Department of Mechanical Engineering on the campus.

log relative energy units (at 450 mμ). A curve relating the log relative energy for each wave length to pupil diameter in millimeters was drawn.

RESULTS. The data obtained on five subjects from the seven wave lengths are summarized in table 2. The pupil diameters represent averages for all subjects of all determinations taken at 10, 20, 30, 45 and 60 seconds after a particular light was thrown in the eye. The light remained 16.6 degrees visual angle throughout the experiments.

When pupil size is plotted against log intensity for each wave length, similar curves are obtained possessing a characteristic sigmoid shape, for white light and for lights of different wave lengths. Average values are used because there are slight differences from reading to reading. The differences are to be accounted for by the fact that not only are there variations in pupil size from individual to individual, but also in the same individual from day to day (Reeves, 1918;

TABLE 1

Relative energies and central wave lengths of each of the Wratten monochromatic filters

FILTER	CENTRAL WAVE LENGTH	RELATIVE ENERGY
	milli-microns	
70	688	13.333
71A	656	6.610
72	613	1.344
73	581	3.236
74	532	1.687
75	491	1.703
76	450	1.000

Laurens, 1923; Crawford, 1936). Since the measurements on each subject were made on different days over a period of several weeks, such variations are to be expected.

The first measurement at each wave length was always the same pupil size as after a 20-minute stay in complete darkness. The first point for wave lengths 688, 656 and 613 milli-microns is an average for all the threshold values determined subjectively. No color could be detected at these intensities by the dark adapted eye. At all other wave lengths, the first point is at an intensity which also does not give a color sensation to the dark adapted eye, but which is slightly above the threshold value for these lights. The pupil diameter at these points was always the same as it was after 20 minutes of dark adaptation.

If all seven curves plotted from the data in table 2 are drawn to the same scale, it is possible to get a picture of the relative stimulating values of each wave length by determining the relative energy for each wave length necessary to cause a constant amount of pupillary constriction. By so doing it is found that 491 milli-microns is the most effective wave length. If all curves are superimposed it is found that the one for this wave length is farthest to the left. In other words, less energy is required to cause a pupillary constriction of, say, 0.5 mm. at this wave length than at any other. A wave length of 532 milli-microns

is slightly less effective in the dark adapted state. The other wave lengths in order of their decreasing effectiveness are: 450, 588, 613, 656 and 688 millimicrons. From a close inspection of the seven log-energy response curves obtained from the data of table 2, it can be seen that these curves maintain, on the whole, their relative positions throughout. There is no indication that the visibility curve, as determined here for a dark adapted eye, ever shows a cone response with a maximum visibility at about 580 milli-microns (Gibson and Tyndall, 1923; Sloan, 1928).

From this information the visibility curve may be determined. Visibility may be defined as the reciprocal of the relative energy necessary, at a given wave length, to produce a given constant physiological response. The visibility curve may be obtained from the various response-log relative energy curves by using the method described by Hecht (1928). One can read off from the curves the abscissa values corresponding to a given response on the ordinate. The reciprocals of these energy values give the relative stimulating capacities of the different

TABLE 2

Pupillary diameters of the human subject at different wavelengths of light at various relative energies

688 MILLIMICRONS		656 MILLIMICRONS		613 MILLIMICRONS		581 MILLIMICRONS		532 MILLIMICRONS		491 MILLIMICRONS		450 MILLIMICRONS	
Log relative energy	Average pupil diameter	Log relative energy	Average pupil diameter	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters
$\bar{2}.4617$	6.2	$\bar{2}.8888$	6.6	$\bar{4}.5675$	6.8	$\bar{4}.9735$	6.9	$\bar{5}.2564$	6.7	$\bar{5}.2523$	6.8	$\bar{5}.4835$	6.5
$\bar{1}.9890$	6.2	$\bar{2}.2937$	6.5	$\bar{4}.1332$	6.9	$\bar{3}.7516$	6.7	4.0345	6.6	$\bar{4}.0304$	6.2	$\bar{4}.2616$	6.4
$\bar{0}.2219$	5.8	$\bar{0}.5266$	5.8	$\bar{2}.9855$	6.8	$\bar{2}.6039$	6.6	$\bar{2}.8868$	6.1	$\bar{2}.8827$	6.3	$\bar{3}.1139$	6.4
1.2213	5.0	0.9066	5.1	$\bar{1}.2184$	5.9	$\bar{0}.8368$	5.8	$\bar{1}.1197$	5.2	$\bar{1}.1156$	5.4	$\bar{1}.3468$	5.5
2.1377	4.5	1.8330	4.8	0.2148	6.5	0.5964	5.0	0.3135	4.4	0.3176	4.2	0.0864	4.5
2.6381	4.4	2.3334	4.4	1.1412	4.7	1.5228	4.6	1.2399	4.0	1.2440	4.1	1.0128	4.0
2.8330	4.0	2.5283	4.1	1.6416	4.4	2.0232	4.0	1.7403	3.6	1.7444	3.7	1.5132	3.8
3.1249	4.0	2.8202	3.6	1.8365	4.1	2.2181	3.8	1.9352	3.5	1.9393	3.5	1.7081	3.5
				2.1284	3.9	2.5100	3.6	2.2271	3.1	2.2312	3.5	2.0000	3.5

parts of the spectrum. The logarithm of the relative energy is plotted against wave length to give the spectral sensibility curve.

The given constant physiological response arbitrarily chosen was a pupillary contraction of 0.5 mm. (cf. Hecht and Pirenne, 1940). In measuring the 0.5 mm. contraction, the starting point was taken as the average size of the completely dark adapted pupil, neglecting the slight differences in these sizes. Since the curves are more or less parallel, a contraction of any amount should give the same results. This has been tried and found to be true.

The spectral sensibility curve determined from the data is shown as the continuous line in figure 3. The position of minimum energy or the maximum visibility is found to be at 510 milli-microns. Visibility falls from this value to low values on either side of the red and violet. This curve is similar to the visibility function for human rod vision as determined by Hecht and Williams (1922) who found the maximum visibility to be at 511 milli-microns, and confirmed by the work of Sloan (1928). The classical dim visibility curve of Hecht and

Williams (1922) is reproduced in figure 3 as the dotted line. It is obvious that both curves agree extremely well, and both represent the spectral sensibility curve of the human eye at low intensities, or rod function. It is definite, from the results, that the rods do play a part in the control of pupillary size. This is in opposition to the view recently advanced by Brown and Page (1939) who have claimed that only the cones influence the movements of the pupil. They arrived at this conclusion merely because of their assumption that the curve of pupillary dilatation in darkness was similar to the curve of subjective dark adaptation. They claimed, from these considerations, that where the pupil showed a rod visibility curve, it resulted "from transmission of excitation from the rods within or outside the macula to optic nerve fibers leading off from cones" which control pupil constriction. This implies that when the cones are stimulated in this manner they cause the pupil to give a false impression of having nervous connections from the rods. This is rather unreasonable, since the cones
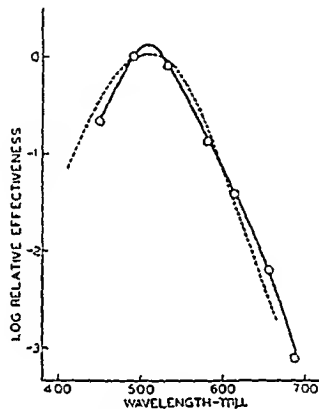
Fig. 3. The human dim vision spectral sensitivity curve (continuous line) as determined by pupil diameter measurements. The dotted line represents the same curve from data determined subjectively by Hecht and Williams (1922). The latter curve has been placed slightly below the former so that the maxima of the two may be compared.

would show similar responses once they are stimulated, no matter whether they are stimulated directly or their corresponding optic nerve fibers are stimulated indirectly.

That the rods, in addition to the cones, influence pupil size is borne out by the previous investigations. Both Engelking (1922) and Hess (1909) showed that the pupil in a totally color blind eye constricted and dilated, although more slowly than in the normal eye. Abelsdorff and Feilchenfeld (1904) showed that when the dark adapted eye was stimulated by a large area, the center of which was black, the pupil reflex was evoked. Finally, we have noticed that a distinct pupillary constriction of about 0.5 mm. in the dark adapted eye is obtained by any wave length of light at intensities which are above the visual threshold but which appear colorless to the subject.

The previous attempt of Laurens (1923) to portray spectral visibility curves from measurements of pupillary size does not meet with the requirements demanded by the classical definition of such curves, since instead of measuring

the relative energy to produce the same physiological effect, he determined the relative amount of physiological effect produced by different wave lengths in an equal energy spectrum. The curve in figure 1 is based upon the classical requirements, and when it is compared with other data, it is seen that it not only coincides with the dim visibility curve of the human eye, but also with the absorption spectrum of visual purple. Thus the evidence that the visibility curve as measured in this investigation is a rod function is strengthened.

SUMMARY

A new infrared photographic method for measuring pupil diameter under any condition of light adaptation is described. It is found that the human dim visibility curve obtained by relating pupil diameter to intensity of different monochromatic lights has its maximum at 510 milli-microns. It is similar in all respects to the classical dim visibility curves and to the absorption spectrum of visual purple. It is therefore believed that the pupil size relative to light stimulation is under the control of fibers activated by the rods of the retina, as well as the cones.

REFERENCES

ABELSDORFF, G. Ztschr. Psychol. u. Physiol. d. Sinnesorg. 22: 81, 1900.
ABELSDORFF, G. UND H. FEILCHENFELD. Ztschr. Psychol. und Physiol. d. Sinnesorg. 34: 111, 1904.
BROWN, R. H. AND H. E. PAGE. J. Exper. Psychol. 25: 347, 1939.
CRAWFORD, B. Proc. Roy. Soc. London 121B: 376, 1936.
CROZIER, W. J. J. Gen. Physiol. 6: 647, 1924.
ENGELKING, E. Klin. Monats. Augenheil. 69: 177, 1922.
FERREE, C. E., G. RAND AND E. T. HARRIS. J. Exper. Psychol. 16: 408, 1933.
GIBSON, K. S. AND E. P. T. TYNDALL. Bur. Stand. Scien. Papers 19: 131, 1923.
GRAHAM, C. H. AND H. K. HARTLINE. J. Gen. Physiol. 18: 917, 1935.
GRAHAM, C. H. AND L. A. RIGGS. J. Gen. Psychol. 12: 279, 1935.
GRUNDFEST, H. J. Gen. Physiol. 15: 307, 1932a; 15: 507, 1932b.
GULLBERG, J. E., J. M. D. OLMSTED AND I. H. WAGMAN. This Journal 122: 160, 1938.
HECHT, S. J. Gen. Physiol. 4: 113, 1921a.
            J. Gen. Physiol. 3: 375, 1921b.
            J. Gen. Physiol. 11: 657, 1928.
HECHT, S., C. HAIG AND A. M. CHASE. J. Gen. Physiol. 20: 831, 1937.
HECHT, S. AND M. H. PIRENNE. J. Gen. Physiol. 23: 709, 1940.
HECHT, S. AND S. SHLAER. J. Opt. Soc. America 28: 269, 1938.
HECHT, S. AND R. E. WILLIAMS. J. Gen. Physiol. 5: 1, 1922.
HESS, C. Arch. Ophthal. 38: 131, 1909.
            Winterstein's Hand. of Verg. Physiol. 4: 555, 1913.
            von Graefe's Arch. fur Ophth. 90: 382, 1915.
LAURENS, H. This Journal 64: 97, 1923.
LUCKIESH, M. AND F. K. Moss. J. Opt. Soc. America 24: 130, 1934.
REEVES, P. Psychol. Rev. 25: 330, 1918.
SACHS, M. Pflüger's Arch. 52: 79, 1892.
SLOAN, L. L. Ph.D. Thesis, Bryn Mawr, 1928.
WAGMAN, I. H. M. A. Thesis, Univ. of Calif. Library, Berkeley, 1937.
WAGMAN, I. H. AND L. M. NATHANSON. Proc. Soc. Exper. Biol. and Med. 49: 466, 1942.

THE EFFECT OF AN INJURED AREA ON THE ELECTRICAL FIELD OF THE HEART BASED ON EXPERIMENTS WITH MODELS[1]

L. N. KATZ, K. JOCHIM AND A. GOLDMAN

From the Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

The monophasic injury curve is recorded during ventricular systole by connecting an electrode on an injured area of the exposed ventricle through a galvanometer to another electrode on another part of the body. The records obtained show the development of a relative positivity of the electrode on the injured area with respect to the other electrode during the period of electrical systole. On occasion, a region of relative negativity may develop simultaneously in the immediate neighborhood of the injured area during electrical systole (1). Not only does the injured region become relatively positive to the second electrode during electrical systole, but it becomes relatively negative during electrical diastole (2, 3). Further, the duration of the monophasic injury current (in bipolar leads) is affected by changing the location of the electrode on uninjured regions but not by changing the location of the injured region (and the electrode placed on it) (4). The duration of the monophasic current is also altered by chemicals when they are placed on the region of the uninjured area of electrode but not when placed on the region of the injured area of electrode (4).

We have pointed out (3) that these observations could best be correlated with the classical membrane theory and that the primary source and sink of the currents was in the electrically polarized membrane. At that time we advanced the view that the cell membranes in the injured region could be considered as being in one of four states: *a*, normal resting polarity across the cell membrane during both diastole and systole—the injured area being unresponsive (5); *b*, no polarity across the cell membranes during either diastole or systole; *c*, partial polarity across the cell membranes only during diastole with no polarity during systole—the region being responsive; or *d*, partial polarity across the cell membranes during both diastole and systole—the injured area being unresponsive. Situation *d* fitted the observations of Eyster, Meek et al. (and our own) on the reversal of the potential of the injured area with respect to that existing in uninjured areas.

We felt that these views might further be clarified by model experiments. For this purpose, the situation of the heart in the body can be imitated in principle by two metal rings placed in a large dish filled with saline in such a way that the dish is completely divided into three compartments. The compartment outside the outer ring represents the body field outside the heart; the compartment between the inner and outer rings represents the cell membrane itself separating the charges on its two sides in the normal polarized resting state of the cell; and the compartment inside the inner ring represents the cell interior. The outer and

inner rings, connected to a source of positive and negative potential represent, respectively the positively charged outer surface and the negatively charged inner surface of the resting cell membrane, according to the classical membrane theory. The fact that the geometric pattern of the syncytium of the heart is infinitely complex does not alter the fact that in its normal resting state the heart is not a simple bipole, but the equivalent of an inner negative pole completely surrounded by an outer positive pole, as represented in simple form by our model. In a three dimensional model this would require one sphere within another with similar complete compartment separation. If such a model is employed and the electrical field determined[2], it should give a clue to the potential differences existing in the body as contributed by the heart when the latter is in its resting polarized state.

We found that no potential difference existed in the outside compartment, the potential being identical throughout with that of the outer of the two metal rings[3]. Hence, during the resting polarized state of the heart existing during diastole, the body will have the same potential as the outside of the polarized cell membrane of the heart syncytium[4]. Similarly it was found that the compartment inside the inner ring had everywhere the same potential as the inner ring, that is to say, the potential inside the syncytial cell of the heart is the same as that on the inner surface of the cell membrane when the cell is in the resting polarized state. It is, however, different from that outside the cell. Between the two rings there is a steep potential gradient going from outer to inner ring, the equipotential lines being circles concentric with the rings.

If in such a model the two rings are discharged so that no potential differences exist, the entire electrical field disappears and all parts of the three compartments are at the same potential, the potential of ground. Thus it is obvious that both in the completely polarized state of rest during diastole, and in the completely depolarized state during systole, a unipolar connection will show the

[2] *Method*. The two metal rings, 3″ and 4″ respectively in diameter and 1″ high are set concentrically in a bakelite base and immersed in the center of a large shallow dish filled with approximately 10 per cent saline. The dish was a varnished wooden tray, 21″ x 24″ x 2″. The voltage supply for the rings and the ring segments is obtained from a potentiometer connected across a source of 110 V.A.C. with center point grounded. An exploring electrode is connected through a pair of headphones to the sliding contact of the potentiometer. Thus, using the headphones as a null detector, isopotential lines may be plotted in the field at any desired intervals, the voltage of the exploring electrode with respect to ground being measured with a high resistance voltmeter for each position of the potentiometer slider. The metal rings rested on the bottom of the tray and emerged from the saline bath.

[3] If the outside field is not connected to ground, this will be some value more positive than ground.

[4] In our experiments each ring was connected to the potentiometer by a wire fastened to one point on the circumference. Thus there was a small circumferential potential gradient along each ring, and consequently a slight potential gradient in the inner and outer compartments. Strictly speaking, the potential of the two rings was +5 and −5 respectively only at the point of connection of the lead wires. If, instead of the rings, the electrodes had consisted of two coaxial cones with the lead wires connected to their apices, there would have been no circumferential potential gradient and hence no gradient in the outer and inner compartments.

distant electrode to be at the same potential as the electrode on or close to the heart's surface. Since the potential of the heart's surface is not the same in diastole as in systole, it is obvious that the potential of the distant electrode also is not unchanged between diastole and systole. The distant electrode cannot, therefore, be considered "indifferent" in the sense that it remains at constant potential. The illusion that distance makes the electrical effect of the heart less is not borne out when comparing the completely polarized with the completely depolarized state in the uninjured heart. These observations further demonstrate the need of signifying precisely what reference potential is intended when speaking of relative positivity or relative negativity. Failure to do this, we fear, has led to some of the apparent contradictions in the literature.

Let us try to imitate in the model the state of affairs outlined in d above, namely, an injured region in which the cell membrane is only partially polarized during the resting polarized state in diastole of the rest of the ventricles, and remaining in this state because it is unresponsive during the systole of the rest of the ventricles. In order to imitate this condition in the simplified model the two rings were split into a smaller and a larger set of segments (see fig. 1), the smaller segments having a lesser potential difference between them than the larger ones. The former set thus represents the injured region which is only partially polarized, and the latter set, the remaining uninjured cell. The three compartments of the field are now no longer completely separated. As figure 1 shows, the entire outside compartment is still positive but not of uniform potential, the potential being greater near the larger segment than near the smaller, and the potential at more distant points being greater on that side of the field in which the larger segments lie than on the side of the smaller segments, the potential difference between the two sides declining as the outer portions of the field are reached. In short, while the entire external compartment is positive with respect to ground, its potential is not uniform, currents will flow, and the region in the neighborhood of the injured part will be relatively negative to the resting polarized uninjured cell (2, 3). The resting injury current is thus due to the incomplete polarization of the injured region.

The inner compartment, equivalent to the cell interior in the heart, will also show potential differences although all parts will be negative with respect to ground. Thus, the resting injury current flows not only in the external field but within the cell syncytium as well.

During electrical systole, the membrane of the uninjured portion of the syncytial cell becomes depolarized, hence the large segments will no longer be charged, while the small segments will retain their charge as in diastole. The field under these circumstances for the model is illustrated in figure 3. The large segments are removed here because, being made of metal, they are a better conductor than the saline bath and so would distort the field.

There is no longer any separation whatever into compartments. Both negative and positive charges are exposed to the entire external field. While in this particular experiment the zero line and negative field are still within the area formerly covered by the inner compartment, this could be changed by using
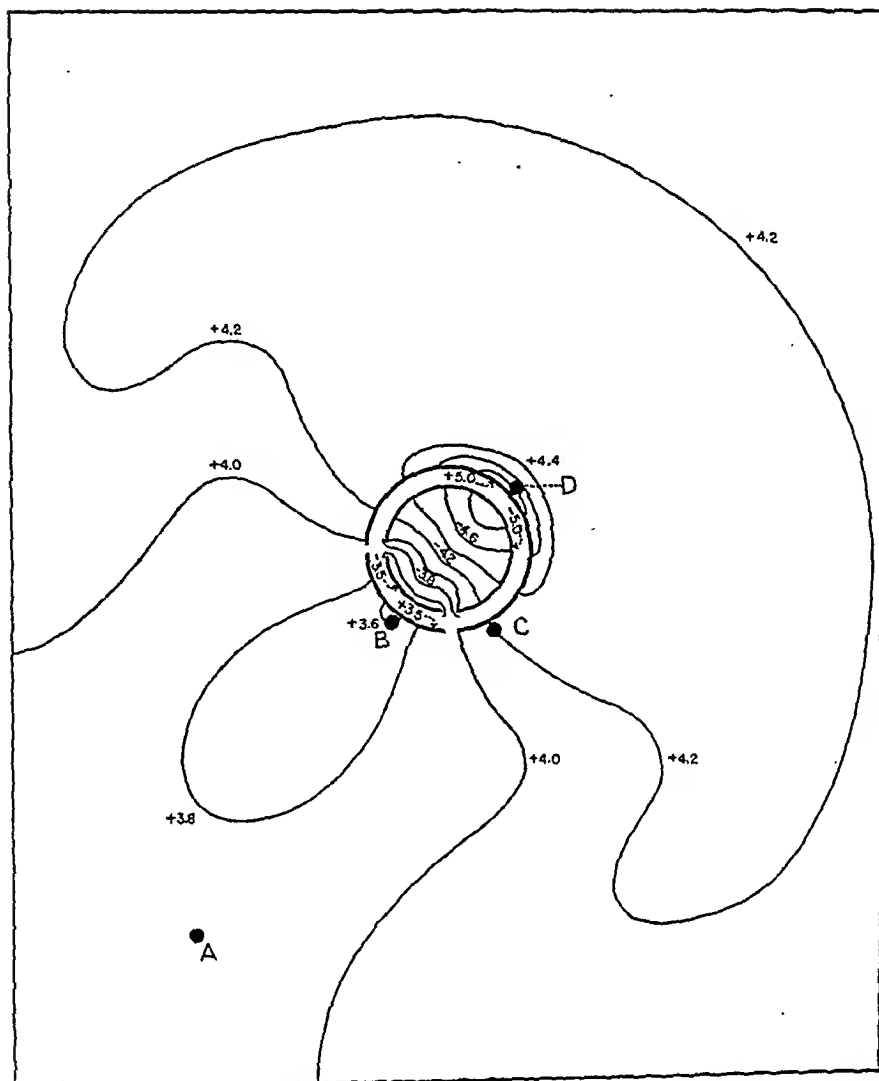
Fig. 1. Distribution of potential around two segmented metal rings, representing the electrical field around an injured heart during the resting polarized state. The pair of smaller segments, representing the partially polarized injured region of the syncytial cell membrane, is connected to a source of smaller voltage than that supplying the larger "completely polarized" uninjured segments. The lines represent equipotential lines are 0.1 volt apart. The figures represent volts. Spot A is at a distant point in the field; B is near the "injured" region; C is near the junction of the "injured" and "uninjured" regions; and D is near the "uninjured" region. Discussed in text.
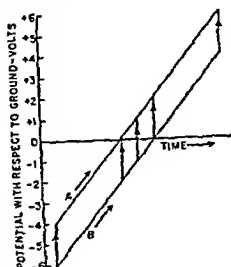
Fig. 2. Diagram illustrating how the potential with respect to ground of two points A and B in a field may change without affecting the potential difference between the two points. Discussed in text.

782
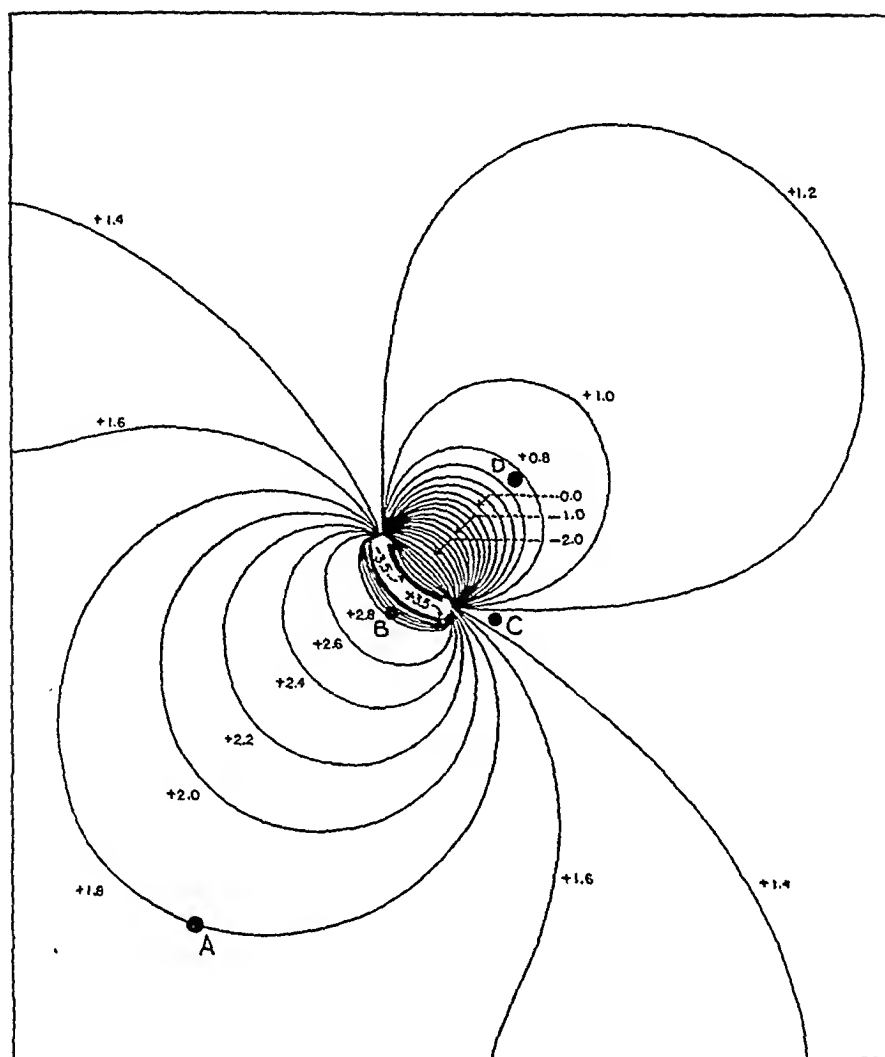
Fig. 3. Distribution of potential around two small metal segments, representing the electrical field of an injured heart during the completely activated state. The large segments of figure 1, now being completely depolarized, have been removed, and the only remaining source of potential is the pair of small segments representing a partially polarized, injured unresponsive region of the cell membrane. Conventions as in figure 1. Discussed in text.
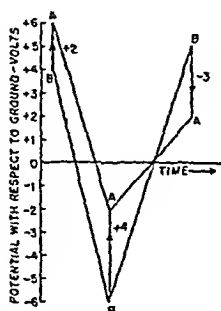
Fig. 4. Diagram illustrating that the direction and magnitude of changes in the potential difference between two points A and B in a field give no indication of the changes in potential of each point with respect to ground. Discussed in text.

smaller segments for the "injured unresponsive region" and then the negative field would extend outside the confines of the "cell".

In the state of systole as depicted in figure 3, the outer portions of the field are still positive with respect to ground, but, unlike the conditions in diastole in figure 1, now the potential is more positive on the side of the field toward the "area of partially polarized unresponsive injury". Thus an electrode on or near the surface of the "injured region" will be relatively positive to any distant electrode (unipolar lead) or to any electrode on an "uninjured responsive part" of the model (bipolar lead). In short, the activity injury current will be the reverse of the resting injury current (2, 3).

This can be illustrated by comparing the potential difference existing in figures 1 and 3 between distant point A and point B close to the "injured region".

In figure 1, $A = +3.9$ v. and $B = +3.6$ v. so that B is 0.3 v. less than A. In figure 3, however, $A = +1.8$ v. and $B = +3.0$ v. so that B is now 1.2 v. more than A. Thus at rest B over the injured region is relatively negative with respect to the distant electrode A. After activation, however, B over the injured region is relatively positive with respect to the distant electrode A. Yet, the change in potential between figures 1 and 3 has been greater in the distant electrode, 3.0 v. $-$ 1.8 v. or a drop of 1.2 v., than in the electrode B 3.6 v. $-$ 3.0 v. or a drop of 0.6 v. Surely the distant electrode is not "indifferent" in this instance.

Similarly a bipolar connection between B and D, the latter on an uninjured region, will show a similar trend. In figure 1, $B = +3.6$ v. and $D = +4.8$ v. so that B is 1.2 v. less than D. In figure 3, B is $+3.0$ v. and D $+0.7$ v. so that B is 2.3 v. more than D. Thus at rest B, over the injured region is relatively negative with respect to D over the uninjured region. After activation, however, B over the injured region is relatively positive with respect to D over the uninjured region. Yet, the change in potential between figures 1 and 3 has been greater over the uninjured, 4.8 v. $-$ 0.7 v. a drop of 4.1 v., than over the injured region, 3.6 v. $-$ 3.0 v. or a drop of 0.6 v.

It is thus obvious that the changes noted by Eyster, Meek et al. (2) which we confirmed (3), do not imply that the injured region is the source for the monophasic action current since the potential in this region is actually little altered during activity of the heart. The results indicate that the change in potential caused by depolarization of the uninjured membrane is responsible for the monophasic action current. This would accord also with the effects we noted on the duration of the monophasic curve.

The observation that a point in the neighborhood of the injured area becomes negative relative to a distant point during systole at the same time that a point on the injured area becomes positive relative to this distant point (1) can also be demonstrated to occur in this model. If, for example, point A in figures 1 and 3 is compared with point C near the smaller "injured" segments, it will be found that unlike B which becomes relatively positive to points A and D during depolarization, spot C becomes relatively negative to these points, viz., in figure 1, $A = +3.9$ v. and $C = +4.2$ v. so that C is 0.3 v. greater than A. At rest, C is positive relative to A while B is negative relative to A. In figure 3, $A = +1.8$ v.

and $C = +1.3$ v., so that C is 0.5 v. less than A. In the activated state, C is relatively negative to A whereas B is relatively positive. Therefore, in the change from the resting to the active state spot C became 0.8 v. relatively negative to A, at the same time that spot B became 1.5 v. relatively positive to A.

This model accounts for all the findings observed in the experiments on the animal. It can therefore be concluded that the monophasic action current in the more complex situation of the cell syncytium is determined primarily by the depolarization and repolarization in the uninjured areas of the heart; the injured area can be conceived as partially depolarized and unresponsive as the result of injury. This state of unchanged polarity in the injured area alters the electrical field during the polarized state of the heart in diastole and the depolarized state in systole from those existing in like periods before injury occurred. Only in this sense can the injury be considered to contribute to the monophasic action current of injury. The actual change in the field, however, between diastole and systole is caused by alterations in the electrical state of the uninjured part of the heart. There is, therefore, no need to accept the contrary interpretation (1, 2) that the monophasic action current is due to potential changes in the injured region. The classical membrane theory adequately accounts for all the facts of the monophasic injury current.

Our results further emphasize one other important fact, namely, that distance from the heart does not exclude the possibility of large changes in potential during the heart cycle. As pointed out in the uninjured cell, the potential in distant regions changes as much as that near the cell between the completely polarized and depolarized states, and in the model with "injury", the distant electrode may show greater changes in potential than regions near the heart.

A similar interpretation of the source of the monophasic action current would apply if the state of the injured cell were either that of a or b above (p. 779). In the former case no change in potential would occur in the injured area since polarization is present to the same extent during systole and diastole; in the latter case no change in potential would occur in the injured region since depolarization persists during the entire heart cycle. The monophasic injury curve is therefore also due in these cases to the process of depolarization and repolarization of the uninjured part of the cell. However, the reversal of relative potential in the neighborhood of the injured area during activation of the heart would not occur in these circumstances. This would also exclude a partially depolarized state in the injured area during diastole, responsive in systole, condition c. However, only in this latter circumstance could the injured region conceivably contribute to the monophasic action potential since its polarity would alter.

It is apparent, therefore, that the whole electrical field of the heart must be examined under varying circumstances in order to elucidate the true origin of the monophasic action current, or of the resting and activity injury currents. This can be aided by model experiments. It cannot be achieved merely by comparing the alterations in potential of two spots in the electrical field in terms relative to each other since this implies that the potential of the spot used as a refer-

ence point is unchanged during the heart cycle with respect to ground potential and this is a fallacious assumption as the present study shows. This may be made clearer by reference to figures 2 and 4. Figure 2 shows that the actual potentials of two points in the field with reference to ground may change without altering the potential difference between them, and figure 4 indicates that there is no parallelism in the magnitude or direction of the actual changes in potential of the two points with respect to ground and the magnitude or direction of the potential difference between them.

These deductions, based on model experiments require confirmation in the animal since conditions in the latter are more complex as regards 1, the relative sizes of the polarized cell membrane, the injured area, and the field 2, the non-homogeneity in conductivity of the field, and 3, the electrical character of the surface of the body, the field boundary. However, we are inclined to view our results with the model as qualitatively valid because they are in accord with observed changes in the animal.

SUMMARY

The electrical field was explored in models made to represent in simple form the syncytial cell of a normal heart and a heart with an injured, unresponsive area. In the "normal heart" model during electrical diastole the entire external or body field was found to be an equipotential region at the same potential as the external surface of the "polarized cell membrane", i.e., positive with respect to ground. During electrical systole when depolarization was complete the entire field was at ground potential.

In the "injured heart" model during electrical diastole the external field was still positive throughout with respect to ground; however, it was no longer an equipotential region because the "injured region" was only partially polarized. This led to the flow of the resting injury current. During electrical systole the potential distribution in the field was altered due to the depolarization of "uninjured" portions of the "cell membrane", while the "injured region", being unresponsive, retained its partial polarization. This gave rise to the activity injury current. It was shown with these models that distant points in the external field may experience large changes in potential with respect to ground between diastole and systole, and so cannot be considered to be indifferent. Further, the resting injury current and the activity injury current and hence the monophasic curve of injury can be explained in these models on the basis of the classical membrane theory. The activity injury current is shown to be due to the depolarization of uninjured regions and not to any process occurring within the injured area.

REFERENCES

(1) WILSON, F. D., F. D. JOHNSTON, I. G. W. HILL AND G. C. GROUT. Proc. Soc. Exper. Biol. and Med. 30: 800, 1933.
(2) EYSTER, J. A. E., W. J. MEEK, H. GOLDBERG AND W. E. GILSON. This Journal 124: 717, 1938.
(3) SUGARMAN, H., L. N. KATZ, A. SANDERS AND K. JOCHIM. This Journal 130: 130, 1940.
(4) JOCHIM, K., L. N. KATZ AND W. MAYNE. This Journal 111: 177, 1935.
(5) ASHMAN, R. AND N. C. WOODY. Proc. Soc. Exper. Biol. and Med. 42: 17, 1939.